GE Healthcare

BPG Columns

Instructions for Use

Important user information

All users must read this entire manual, along with the BPG Columns Maintenance Guide, to fully understand the safe use of BPG columns.

Amersham Biosciences AB, which was previously known as Amersham Pharmacia Biotech AB, changed its name to the current name on 17th October 2001.

CE Certifying

This product meets all requirements of applicable CE-directives.

The **CE** symbol and corresponding declaration of conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other CE-marked Amersham Biosciences instruments, or
- connected to other products recommended or described in this manual, and
- used in the same state as it was delivered from Amersham Biosciences, except for alterations described in this manual

Declaration of conformity

A Declaration of conformity stating European directives and harmonized standards valid for this type of equipment is included separately with each unit.

Safety Precautions

IMPORTANT!
Whenever the column is used, always keep the Instructions for Use manual for the column on hand.

IMPORTANT!
The end user must ensure that all installation, maintenance, operation and inspection is carried out by qualified personnel who are adequately trained and understand the operating instructions.

WARNING! Always use protective clothing appropriate with current application to ensure personal safety during operation.

WARNING! Pay extra attention when working close to the column.

WARNING! There should NEVER be air or gas under pressure in the column.

WARNING! The working pressure of the column should never exceed the design pressure, otherwise there is a risk of personal injury and damage to the column. A suitable safety device must be fitted.

WARNING! The working temperature range of the column should never fall outside the design temperature range.

WARNING! Do not use other chemicals than those proven not harmful to the wetted parts of the column.

WARNING! The wheel brakes must be activated at all times during operation or storage.

WARNING! Pay extra attention when moving the column to ensure that the column does not tip.

CAUTION! Ensure that any water used with the column is particulate-free, since particles can block and damage the meshes.

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1 Introduction

1.1 General

BPG columns are intended for industrial applications that demand high standards of hygiene. The columns are available for chromatographic separation media volumes up to 131 liters, and permit the use of high flow rates.

When BPG columns are used in conjunction with BioProcess Media, such as Sepharose Fast Flow and Sephacryl High Resolution, the most stringent regulatory demands can be satisfied. The materials from which BPG columns are manufactured have been carefully selected to resist sanitizing and cleaning agents such as sodium hydroxide and ethanol.

The pressure specifications and low flow resistance make these columns suitable for use with BioProcess Media in ion exchange, hydrophobic interaction, affinity and gel filtration chromatography. Desalting with Sephadex G-25 Coarse, Medium, Fine and SuperFine grades can also be used effectively. The flow distribution system has been developed to match the high performance of Amersham Biosciences media.

All BPG columns are supplied complete with an adaptor. Packing devices are available for applications requiring extended bed heights.

Column assembly and column packing protocols for BioProcess Media are also described in this manual. A special section in the BPG Columns Maintenance Guide (Chapter 1) is dedicated to column maintenance protocols applicable to individual process requirements.

The three major assemblies that comprise the column (adaptor, column components, and stand) are shown in the fold-out exploded diagrams at the back of this manual.

The basic components of the column are:

- Glass tube
- Flange
- O-rings
- Filter nets
- Support nets
- End-piece
- Rods

The rods hold the flange and end-piece to the glass tube which is sealed by O-rings. The O-rings effectively isolate the glass tube from direct contact with the stainless steel components. The adaptor top plate assembly and adjuster nut are clamped or bolted to the column flange. The adjuster nut is allowed to rotate on a bushing ring in the top plate, altering the height of the adaptor plate in the column tube. The adaptor O-ring forms a seal between the adaptor and the glass column when it is compressed by the sealing unit. This is controlled by the seal adjuster knob. The stand is bolted to the column end-piece and is fitted with wheels with foot-operated breaks.

Note: *BPG 100 columns have adjustable feet. Wheels can be ordered as an option.*

BPG columns must not be used for any other purpose, for example:

- Storage tank for chemicals, etc.
- General pressure vessel.
- Fermentation vessel.
- Gas storage.

1.2 Labelling of the column

The CE mark on the identification label shows that the column fulfills the requirements of the European pressure equipment directive (PED) 97/23/EC and/or the directive for equipment in potentially explosive atmospheres (ATEX100) 94/9/EC. Columns where the product of volume and allowed pressure is too small must not be CE marked according to PED. Equipment delivered outside the European Community might not be CE marked.

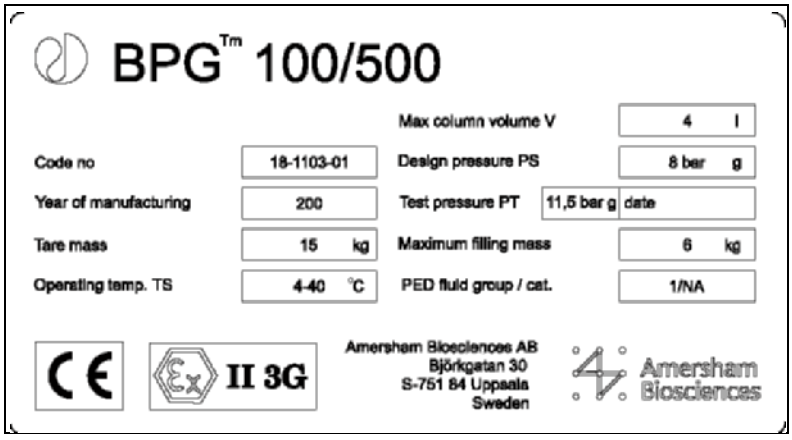

Fig 1-1. The BPG column rating plate.

1.3 Safety

1.3.1 General

Information given in this Instructions for Use is suggested best working practice and shall in no way take precedence over individual responsibilities or local regulations. Great effort has been placed on the design and manufacture of the various parts of the equipment so that it will comply with all applicable safety aspects for this type of equipment. During the operation and during other work with a pressurized vessel, it is always each individual's responsibility to consider:

- Their own and others' personal safety.
- The safety of the equipment through correct use in accordance with the descriptions and instructions given in this Instructions for Use.

1.3.2 Warnings

WARNING! The Warning symbol highlights instructions that must be strictly followed in order to avoid personal injury. Do not proceed until the instructions are clearly understood and all stated conditions are met.

1.3.3 Caution notices

CAUTION! The Caution sign highlights instructions or conditions that must be followed to avoid damage to the product or to other equipment. Do not proceed until the instructions are clearly understood and all stated conditions are met.

1.3.4 Notes

Note: *The Note sign is used to indicate information important for trouble-free and optimal use of the product.*

1.3.5 Chemical resistance

Specifications including chemical resistance are included in Chapter 3 of this Instructions for Use.

1.3.6 Use in potentially explosive atmosphere

BPG columns do not contain any electrical circuits. However, the following precautions should always be observed:

- Avoid discharge from static electricity by grounding the column. Use grounding (earthing) wire connected to the plant grounding network A grounding kit is available as an accessory.
- Only tools for use in potentially explosive atmospheres should be used for operation and maintenance.
- Always use suitable clothing when working in potentially explosive environments.

EX marking
The EX code on the identification label defines the level of explosion protection of the equipment. The EX category of the equipment must be in conformity with the protection level of the room where it is to be used.

Ex II 3 G
Equipment in this category is designed to be capable of functioning in conformity with the operating parameters established by the manufacturer and ensuring a normal level of protection. Equipment in this category is intended for use in areas in which explosive atmospheres caused by gases, vapors or mists are unlikely to occur or, if they do occur, are likely to do so only infrequently and for a short period only.

1.4 Approved operators

A BPG column is a piece of equipment that can be compared with a pressure vessel with a variable volume. Operators should have experience of working with pressure vessels as well as with equipment that provides pressurized media for axial compression packing control. To use the column safely and correctly, the operator must have read, understood and be fully acquainted with the Instructions for Use in general and the safety instructions in particular. Improper operation of the column can result in property damage as well as personal injury.

1.5 Storage

The column must be stored at design-specified temperature and must not be exposed to extreme temperature fluctuations. 20% ethanol is a recommended storage buffer.

1.6 Disposal and recycling

When the column has reached the end of it's life cycle, it should be handled according to current laws and regulations regarding environmental aspects of recycling and disposal of industrial equipment. This is the responsibility of the end user or equivalent. Contact your local authorities if you have any questions regarding the environmental aspects or the laws in force for recycling or disposing of industrial equipment of this type.

1.6.1 Materials and matter

For disposal purposes, materials are generally divided into the following categories:

- Flammable
- Wood
- Compound (e.g., coated with plastic or metal foil)
- Paper
- Non-flammable
- Metal

Note: *Metals that are hazardous or contain hazardous matter (e.g., lead, cadmium, mercury, etc...) must be separated and disposed of in a controlled way.*

- Plastics, separated into hard (hardened) and soft (thermo) plastics
- Composting material (all materials/matter that is converted to soil in the near future)
- Depositing material (a small number of materials/matter that are not suitable for recycling must be deposited. Places for depositing can be located by contacting local authorities.)

1.6.2 BPG columns

Before the column equipment is disposed of, the following steps must be carried out:

- The column should be emptied, cleaned, and sterilized.
- For non-flammable metals such as stainless steel, contact a well-recognized local scrapping company.

1.7 Purpose of this manual

This manual is designed as a general introduction and guide to column operation. Specifically, the manual will cover:

- Introduction (Chapter 1)
- Unpacking the column (Chapter 2)
- The BPG column (Chapter 3)
- Installation and leakage test (Chapter 4)
- Operation (Chapter 5)
- Abbreviations (Chapter 6)

The accompanying manual, "BPG Columns Maintenance Guide", covers the following topics:

- Maintenance and service (Chapter 1)
- Trouble-shooting (Chapter 2)
- Ordering information (Chapter 3)

2 Unpacking the column

Unpack the equipment carefully and check it for signs of damage that may have occurred during transportation.

Table 2-1 below gives the code numbers for the column tubes and stands for the different column diameters - check the code numbers of the new equipment to assure that it matches.

Table 2-1. Code numbers for column tubes and stands..

Column diameter (mm)	Column tube length (mm)			Stand
	500	750	950	
100	18-1103-01	18-1103-02	18-1103-03	18-1031-10
140	18-1113-08	44-7320-47	18-1113-09	18-1031-20
200	18-1103-11	18-1103-12	18-1103-13	18-1031-20
300	18-1103-21	18-1103-22	18-1103-23	-
450	18-1103-71	18-1103-72	18-1103-73*	-

* The tube height of the BPG 450 column is 1000 mm.

Note: *Both the stand and the column must be ordered to obtain a complete column set-up for BPG 100, 140 and 200.*

Check that all of the components listed in Table 2-2 below are present.

Table 2-2. Quantity of components supplied with BPG columns.

Designation	Quantity				
	BPG 100	BPG 140	BPG 200	BPG 300	BPG 450
Blind flange with gasket, EPDM	2	2	2	2	2
Clamp 23 mm, 51 mm	2	2	2	2	2
Stand	-	-	-	1	1
23 µm nets	2	2	2	2	2
Bolts for attaching the stand to the column	-	-	-	6	4
Instructions for use/Maintenance Manuals (each)	1	1	1	1	1

3 The BPG column

This chapter provides a general background on the specifications and characteristics of BPG columns.

3.1 Specifications and characteristics

Table 3-1. Primary specifications and characteristics of BPG columns.

Column diameter (mm)	Cross - sectional area (cm^2)	Tube height (cm)	Bed height [1,3] (cm) min max	Bed volume [1,3] (liters) min max	Max pressure[1] (bar)	Total weight (kg)	Adaptor weight (kg)	Overall dimensions (cm) DxWxH
100	79	50	0 26 (48)	0.0 2.0 (3.8)	8	15	7	48x48x127
100	79	75	25 41 (66)	2.0 3.2 (5.2)	8	16	7	48x48x152
100	79	95	45 54 (83)[4]	3.5 4.2 (6.6)	8	17	7	48x48x172
140	154	50	0 26 (48)	0.0 4.0 (7.4)	6	25	11	59x59x127
140	154	75	25 41 (66)	3.9 6.3 (10.2)	6	26	11	59x59x152
140	154	95	45 54 (83)	6.9 8.3 (12.8)	6	27	11	59x59x172
200	314	50	0 26 (48)	0.0 8.2 (15.1)	6	34	13	59x59x127
200	314	75	25 41 (66)	7.3 12.9 (20.7)	6	36	13	59x59x152
200	314	95	45 54 (83)[4]	14.1 16.9 (26.1)	6	39	13	59x59x172
296	688	50	0 26 (48)	0.0 17.9 (33.0)	4	68	29	69x69x133
296	688	75	25 41 (66)	17.2 28.7 (45.4)	4	73	29	69x69x158
296	688	95	45 54 (83)[4]	31.0 37.2 (57.1)	4	78	29	69x69x178
446	1562	50	3 23 (42)	4.7 35.9 (65.6)	2.5	200	100	80x80x140
446	1562	75	22 39 (58)	34.4 60.9 (90.6)	2.5	215	100	80x80x165
446	1562	100	47 58 (83)[4]	73.4 90.6 (131.2)[5]	2.5	230	100	80x80x190

[1] Maximum bed volumes and bed heights are based on a slurry concentration of 75% and a packing compression of 15%. The compression is the difference in volume between a sedimented bed and a bed under pressure.
[2] Use a manometer to monitor the pressure (to order, see Chap. 3 in the BPG Maintenance Guide).
[3] The figures in parentheses are achievable using a packing extension.
[4] Maximum bed volume and bed height are based on a slurry concentration of 80% and a packing compression of 15%.
[5] Maximum bed volume and bed height are based on a slurry concentration of 85% and a packing compression of 15%.

'The operating temperature for BPG columns is 4°C to 40°C.

Table 3-2. Inlet and outlet dimensions on BPG columns..

	Column size				
	100	**140**	**200**	**300**	**450**
I/O dimensions, mm	4	6	6	10	12

3.2 Column materials

The materials used in the construction of BPG columns have been carefully selected for their compatibility with the solvents most commonly used in chromatography separations of biomolecules, column maintenance and cleaning processes. The polymeric materials have been tested for biological reactivity according to USP XXIII. The materials have also been subjected to in vitro testing according to the "Elution Test" and in vivo testing according to the "Systemic Injection Test" and the "Intracutaneous Test". Extracts of 0.9% NaCl and sesame oil have been used as samples in the in vivo tests. All materials meet the requirements as described in USP XXIII, Class VI.

The components of the column may be considered to contain wet parts, those coming into contact with process liquids, and dry parts. All materials of construction are made of high grade materials which meet the requirements of the industrial production environment. Tables A-1, A-2 and A-3 in the Appendix show the components of the columns and identify the materials from which the wet and dry parts of each component are manufactured.

3.3 Chemical resistance

Table 3-3 is intended as a guide to the solvent resistance of the materials used in BPG columns. The information has been compiled from published material from several sources, not from individual tests on the components of the column. Consequently, the operator should use this list only as a guide to the level of chemical resistance exhibited by each material. It should be noted that the effects of a chemical will be more severe at higher temperatures and pressures, and that combined effects have not been taken into consideration.

In general, the use of the following chemicals in BPG columns should be avoided:

- Extreme oxidizers (such as peroxides)
- Halogenated compounds
- Chlorinated solvents (such as methylene chloride)
- Esters (such as acetates)
- Aromatic hydrocarbons (such as toluene)

- High concentration of strong acids
- Chloroform
- THF
- Toluene
- Methylenedichloride

Note: *Avoid salt in combination with pH below 4. Always wash the column with at least two column volumes of pH-neutral solution between use of salt and low pH buffers.*

Table 3-3. Chemical resistance table of materials in BPG columns.

Substance	Concentration by volume	60-90 days[1]
Acetic acid	10%	see note 7
Acetic acid	25%	see note 7
Acetonitrile	5%	see note 2
Acetonitrile	50%	see notes 3, 4
Acetone	10%	OK
Cyclohexane	100%	see notes 3, 4
Ethanol	100%	see note 2
Ethyl acetate	100%	see note 4
Ethylene glycol	50%	OK
Glycerol	100%	OK
Hexane	100%	see notes 2, 7
Hydrochloric acid	0.1 M	see notes 6, 7
Isopropyl alcohol	100%	see note 2
Methanol	100%	OK
Nitric acid	0.1 M	see note 7
n-Propanol	100%	OK
Sodium chloride	2 M	see note 5
Sodium hydroxide	2 M	OK
Trifluoroacetic acid	0.1%	see notes 7, 8
Triton ® X-100	100%	OK
Tween/Tri-n-butyl phosphate	1% / 0.3%	OK
UREA	8 M	OK

[1] The test does not include PVC tubing.
[2] EPDM rubber changes characteristics. For repetitive and long term use, use FEP/PFR/PTFE O-rings and gaskets.

[3] Change to FEP/PFR/PTFE O-rings and gaskets.
[4] Polypropylene plastic resistance is fair.
[5] Can be used under normal running conditions. Do not use NaCl in storage solutions. Please note that NaCl can cause corrosion on stainless steel in acid solutions (pH below 4.0).
[6] Not longer than 4 hours.
[7] Not recommended to be used with PA nets.
[8] FEP changes characteristics. Thus, use EPDM rubber.

3.4 *Pressure/flow rate curves*

In general, theoretical pressure/flow rate curves are very difficult to develop. Most accurate pressure/flow rate curves are obtained by experimental methods. Figure 3-1 below shows the typical set-up used in Amersham Biosciences laboratories to generate pressure/flow rate curves.

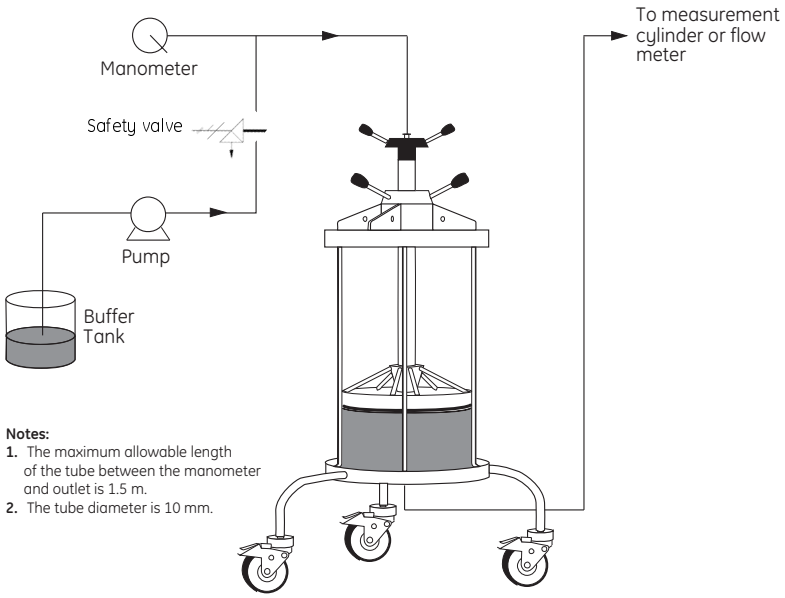
To measurement cylinder or flow meter
Manometer
Safety valve
Pump
Buffer Tank
Notes:
1. The maximum allowable length of the tube between the manometer and outlet is 1.5 m.
2. The tube diameter is 10 mm.

Fig 3-1. Typical set-up for determining pressure/flow rate curves. NOTE: The column contains only buffer. The outflow must be at the same level as the manometer.

3.4.1 Pressure/flow rate curves for empty columns

Figures 3-2 and 3-3 show the pressure/flow rate curves for empty BPG columns, obtained using the method given in section 5.1.3 of this manual and the set-up shown above.

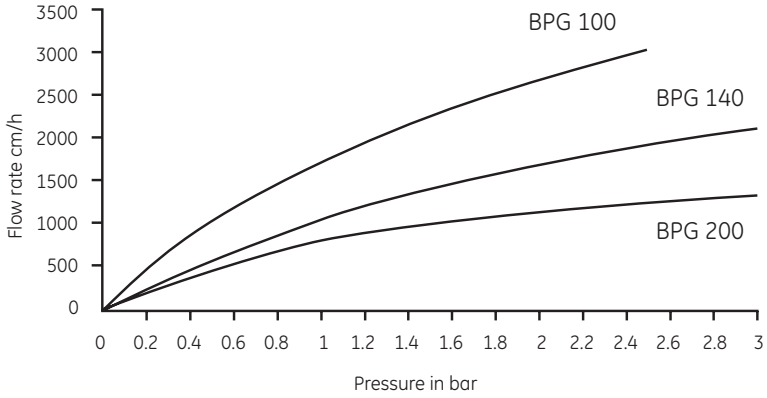

Fig 3-2. Examples of pressure/flow rate curves for empty BPG 100, 140 and 200 columns.

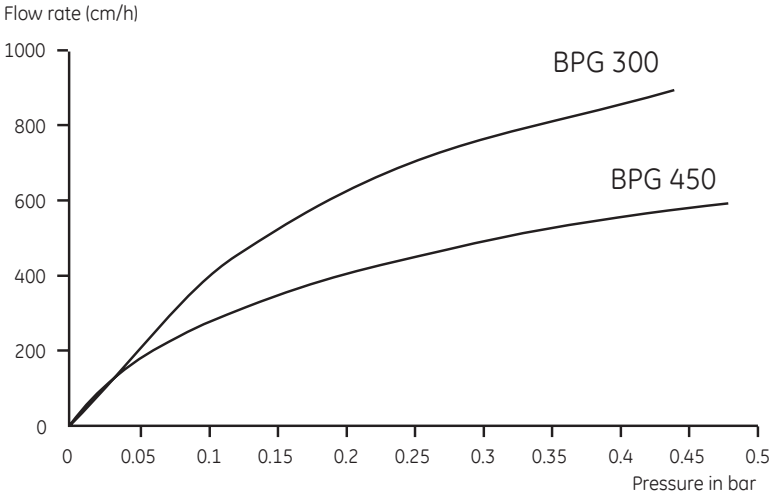

Fig 3-3. Pressure/flow rate curves for empty BPG 300 and 450 columns.

3.4.2 Pressure/flow rate curves for packed columns

Figures 3-4 to 3-6 show the pressure/flow rate curves for packed BPG columns, obtained using the method given in section 5.1.5 and the set-up shown in Fig. 3-1.

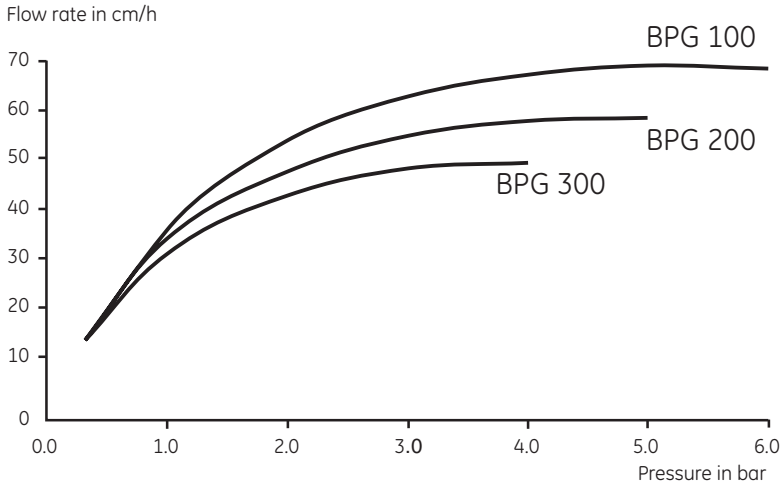

Fig 3-4. Pressure/flow rate curve for Superdex 200 prep grade in BPG columns. Bed height 60 cm.

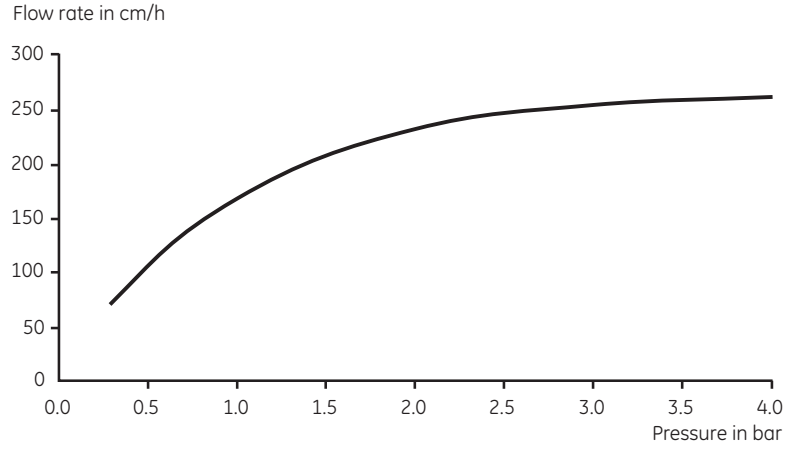

Fig 3-5. Pressure/flow rate curve for Q Sepharose High Performance in BPG 300/500. Bed height 10 cm.

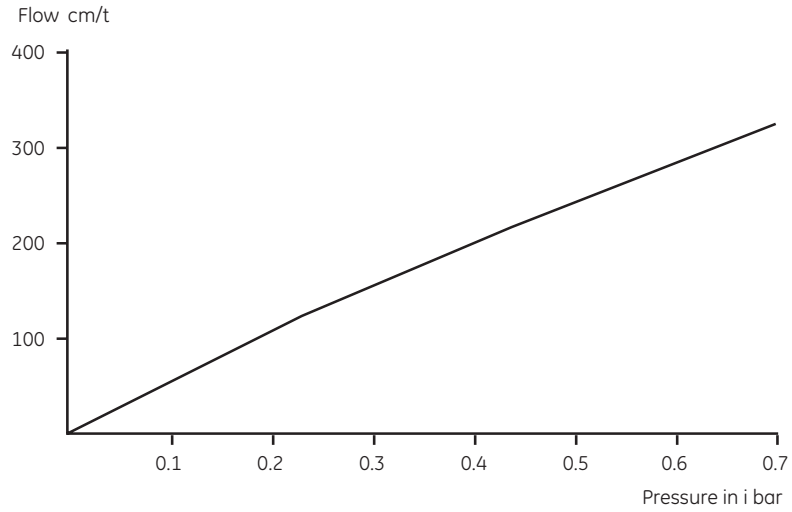

Fig 3-6. Pressure/flow rate curve for a BPG 450 column packed with Q Sepharose Fast Flow to a bed height of 15 cm.

4 Installation and leakage test

All BPG columns have sanitary clamp fittings as connectors.

Caution! Only use hoses and clamps approved for the maximum pressure of the column and resistant to the chemicals intended to be used.

Caution! Place the column in an out of the way area to protect it from being unintentionally moved or run into. Be careful not to step on, or fall over, hoses placed on the floor.

4.1 Disassembly and assembly

Disassemble and assemble the columns and adaptors in accordance with the instructions in the sections below. Refer to Tables A-1 through A-3 and Figures A-1 through A-5 for item identification (these tables and figures are located at the back of this manual). Numbers in parentheses () throughout the sections below refer to item numbers on the associated figures and tables at the back of this manual.

WARNING! After reassembling the column, ensure that the column is tight and not leaking.

4.1.1 BPG 100, 140 and 200

Column disassembly

To disassemble the BPG 100, 140 and 200 columns, refer to Table A-1 and Figures A-1 and A-2 at the back of this manual and proceed as follows:.

1 Remove the domed nuts (18) and washers (14) that secure the adaptor to the flange (10).

WARNING! If the adaptor O-ring seal is dry, it may be damaged by forced movement of the adaptor. Wet the O-ring with water or 20% ethanol solution. Do NOT grease the O-ring.

2 Unscrew the adjusting knob (17) several turns to loosen the adaptor O-ring seal (28/29) and remove the adaptor.

3 Using a socket wrench, and in the sequence shown in Fig. 4-1, remove the nuts (8) and cup spring washers (9) that secure the support rods (2) to the flange (10).

WARNING! Do not loosen each nut completely in one movement. Loosen the nuts step-by-step in the sequence shown in Figure 4-1. Three to four turns per nut is enough to loosen and remove the nuts in the correct manner. A crescent wrench should be used on the flattened areas on the rods to prevent the rods from unscrewing.

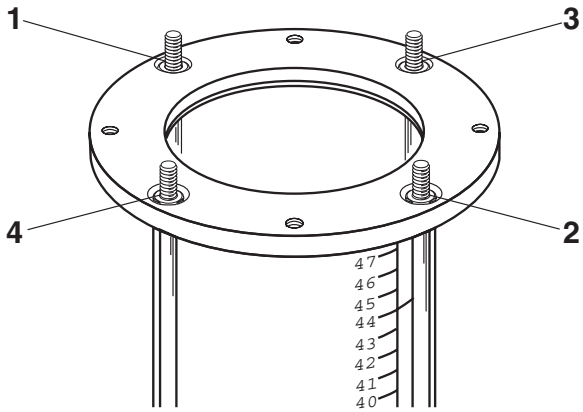

Fig 4-1. Nut removal and tightening sequence.

WARNING! When the flange is removed, the column tube is unsupported. Care should be taken to ensure that the column tube does not tip over.

4 Carefully remove the flange (10).

5 Using an adjustable wrench, unscrew the support rods (2) from the end-piece (12).

6 Lift off the glass tube (1). Carefully place the glass tube on a secure surface. If the O-ring adheres to the glass tube or the end-piece, wet the O-ring with 20% ethanol and remove it.

7 Using a wrench, remove the bolts (13) and washers (14) securing the end-piece (12) to the stand (15). Separate the end-piece and stand.

Column assembly

To assemble BPG 100, 140 and 200 columns, refer to Table A-1 and Figure A-2 at the back of this manual. Proceed as follows:

Note: *Wet the net with 20% ethanol to eliminate trapped air.*

1 Referring to Figure 4-2 below, ensure that the following items are positioned correctly in the end-piece (12):

- Support net (5)
- Filter net (4)
- PTFE guide ring (3)
- O-ring (6/7)

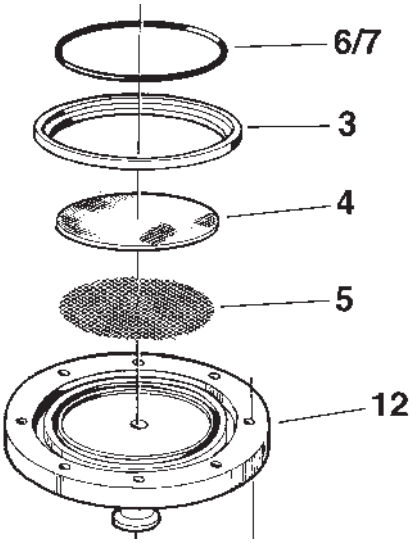

Fig 4-2. End-piece component location on BPG 100, 140 and 200 columns.

2 Secure the end-piece (12) to the stand (15) using the bolts (13) and washers (14).

3 Carefully place the glass tube (1) on the O-ring (6/7) in the endpiece (12). Ensure that the glass tube does not touch any stainless steel. Align the tube with the filter net (4).

4 Screw the support rods (2) into the end-piece (12).

5 Position the flange (10) so that the support rods (2) fit into the appropriate holes. Make sure that the O-ring (6/7) on the flange is aligned with the glass tube. The glass tube should not touch any stainless steel: the glass tube may crack as a result.

6 Align the inner surface of the glass tube with the inner surface of the flange.

7 Mount the cup spring washers (9) and the nuts (8) on the support rods (2) and tighten them by hand.

8 Set a torque wrench to the specifications set out in Table 4-1 below and tighten the nuts (8) and cup spring washers (9) to secure the support rods (2) to the flange (10). Follow the sequence shown in Figure 4-1 (that is, tighten the nuts in a diametrically opposite pattern).

WARNING! The glass tube will crack if too much force is exerted when tightening the four nuts on the support rods - the rods that secure the flange and the end-piece to the glass column. Always use the torque wrench when tightening these nuts.

Table 4-1. Torque wrench settings for BPG 100, 140 and 200 columns.

	BPG Column		
	100	**140**	**200**
Moment (Nm)	4	5	5

9 The column is now ready for use and the adaptor can be inserted.

Adaptor disassembly

To disassemble the adaptor on BPG 100, 140 and 200 columns, refer to Table A-1 and Figure A-1 and proceed as follows:

1 Remove the filter net (36) by bending it carefully. Remove the support net (35). Be careful not to scratch the adaptor as this may cause leakage.
2 Remove the stopper (25) from the top plate (21) by removing the bolts (24).
3 Release the sealing unit (27) and inner adaptor tube (30) by unscrewing the bolts (26) on the upper ring of the sealing unit.
4 Carefully remove the adjusting knob (17). This releases the tension on the spring (33) situated under the inner adaptor tube (30).
5 Remove the top-plate assembly (19-23) and separate it from the outer adaptor (31) by turning it in a clockwise direction.
6 Remove the sealing unit (27) and the adaptor O-ring (28/29).
7 Remove the outer adaptor tube (31), the inner adaptor tube (30) and spring (33) by removing the screws (32).
8 The top plate assembly (19-23) can be disassembled by removing the screws (23).

This completes the adaptor disassembly procedure.

Adaptor assembly

To assemble the adaptor on BPG 100, 140 and 200 columns, refer to Figure A-1 (and Table 4-1 for torque wrench settings) and proceed as follows:

1 Position the bushing ring (20) in the recess in the top plate (21).
2 Using the screws (23) secure the adjuster nut insert (22) to adjuster nut body (19) through the top plate (21) so that the adjuster nut body turns on the bushing ring (20).
3 Slide the spring (33), inner adaptor tube (30) and outer adaptor tube (31) onto the adaptor plate (34).
4 Fasten the outer adaptor tube (31) to the adaptor plate (34) using the screws (32).
5 Slide the sealing unit (27) onto the adaptor tube (31).
6 In a counter clockwise direction, screw down the top plate assembly (19-23) 5 cm onto the outer adaptor tube (31).
7 Turn the adjusting knob (17) clockwise onto the top of the adaptor until the holes in the inner adjusting tube (30) are level with the holes in the outer adaptor tube (31).

8 Finger-tighten down the screws (26) into the upper half of the sealing unit (27), making sure they remain level and are aligned correctly with the holes, until 1-2 mm the screw thread shows. Finally, tighten the screws with a ring spanner.

9 Mount the stopper (25) onto the plate (21) with the screws (24).

10 Mount the adaptor O-ring (28/29) on the adaptor plate (34).

11 Wet the filter net (36) with 20% ethanol. Mount the support net (35) and the filter net (36) on the adaptor plate (34). It may help to warm the filter net in water to facilitate mounting on the adaptor.

This completes the adaptor assembly procedure.

Mounting an extension tube (packing device)

The extension tube is assembled when delivered. Disassembly/assembly of the extension tube is done the same way as for the column, with the exception of the stand directives.

To mount an extension tube onto BPG 100, 140 and 200 proceed as follows:

1 Remove the adaptor from the column and place the extension tube onto the upper flange (10) so that the column rods fit into the open holes in the flange (Fig. 4-3 below) on the extension tube. Make sure that the thin O-ring is positioned properly in the flange.

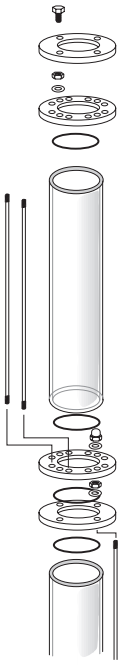

Fig 4-3. Packing device component location.

2 Use the washers and domed nuts from the column lid (adaptor) to secure the extension tube to the column.

The extended column is now ready for the first packing step in two-step packing procedures. The bolts and washers supplied with the extension tube are used to mount the adaptor onto the extension.

Note: *Do not pack the column to bed heights higher than the length of the column, i.e. the adaptor should seal against the column wall, NOT against the extension tube wall. A tube length of 10 cm is required when installing the adaptor onto the column tube if the packing extension is removed.*

4.1.2 BPG 300

Column disassembly

To disassemble the BPG 300 column, refer to Table A-2 and Figures A-3 and A-4 at the back of this manual and proceed as follows:

1 Loosen one of the nuts (4) on the top plate clamp (1) that secures the adaptor assembly to the flange (5) and remove the clamp.

WARNING! If the adaptor O-ring seal is dry, forced movement of the adaptor can damage it. Wet the O-ring with water or 20% ethanol solution. DO NOT grease the O-ring.

2 Unscrew the seal adjuster (23) a few turns, sufficient only to loosen the adaptor O-ring seal (33/34). Remove the adaptor without letting the stainless steel touch the glass.

3 Using a wrench, and in the sequence shown in Fig. 4-4, remove the nuts (2) and cup spring washers (3) that secure the support rods (8) to the flange (5).

CAUTION! Do not loosen each nut completely in one movement. Loosen the nuts step-by-step in the sequence shown in Figure 4-4. Three to four turns per nut is enough to loosen and remove the nuts in the correct manner. An adjustable spanner should be used on the flattened areas on the rods to prevent the rods from unscrewing.

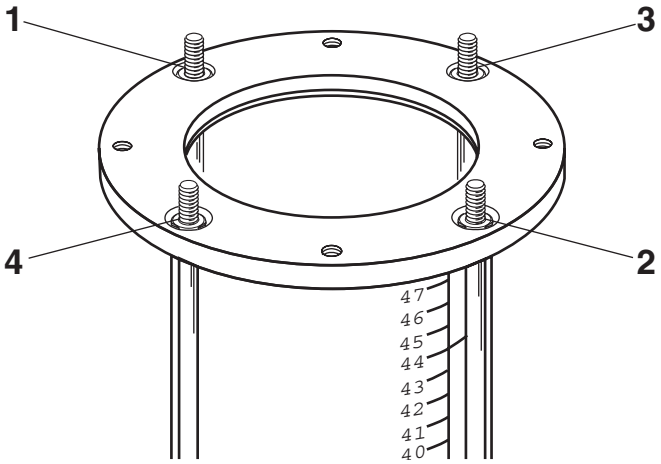

Fig 4-4. Nut removal and tightening sequence.

CAUTION! When the flange is removed, the column tube is unsupported. Care should be taken to ensure that the column tube does not overbalance/fall over.

4 Carefully remove the flange (5).

5 Using an adjustable spanner, unscrew the support rods (8) from the end-piece (14).

6 Lift off the glass tube (9). Carefully place the glass tube on a secure surface. If the O-ring adheres to the glass tube or the end-piece, wet the O-ring with 20% ethanol solution and then remove it.

7 Using a ring spanner, remove the bolts (21) and washers (20) securing the end-piece (14) to the stand (19). Separate the end-piece and stand.

Column assembly

To assemble the BPG 300 column, refer to Figure A-4 and proceed as follows:

Note: *Wet the net with 20% ethanol to eliminate trapped air.*

1 Referring to Figure 4-5, ensure that the following items are positioned correctly in the end-piece (14):

- Distribution plate (13; The profiled side should face the end-piece, and the flat side should face the support net)
- Support net (12)
- Net (10/11)
- O-ring (6/7)

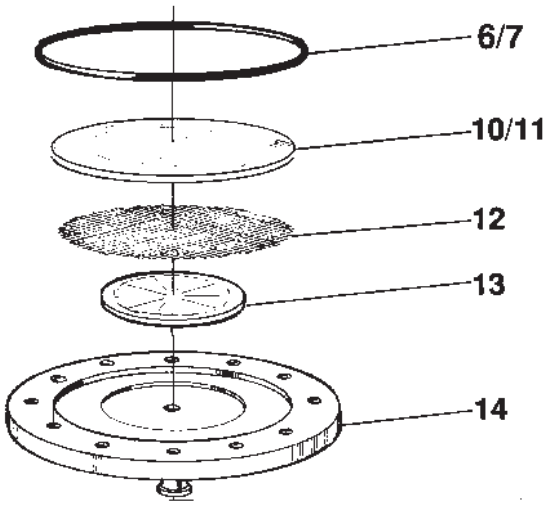

Fig 4-5. End-piece component location for BPG 300 columns.

2 Secure the end-piece (14) to the stand (19) using the bolts (21) and washers (20).

3 Carefully place the glass tube (9) on the O-ring (6/7) in the endpiece (14). Ensure that the glass tube does not touch the stainless steel. Align the glass tube with the bottom filter net (10/11).

4 Screw the support rods (8) into the end-piece (14).

5 Position the flange (5) so that the support rods (8) fit into the appropriate holes. Make sure that the O-ring (6/7) on the flange is aligned with the glass tube. The glass tube should not touch the stainless steel: the glass tube may crack as a result.

6 Align the inner surface of the glass tube with the inner surface of the flange.

WARNING! The glass tube will crack if too much pressure is exerted when tightening the four nuts on the support rods - the rods that secure the flange and the end-piece to the glass column. Always use the torque wrench when tightening these nuts. Refer to Table 4-2 for torque settings.

7 Locate the cup spring washers (3) and the nuts (2) on the support rods (8) and finger tighten the nuts.

8 Set the torque wrench in accordance with Table 4-2 below, and tighten the nuts (2) in the sequence shown in Fig. 4-4. Tighten the nuts stepwise to avoid uneven mechanical pressure on the glass tube. Three to four turns per nut are recommended.

Table 4-2. Torque wrench settings for BPG 300 columns.

Item (Refer to Fig. A-4 for location)	Torque wrench setting
Rod nuts (2)	6 Nm
Top plate clamp nuts (4)	6.5 Nm

The column is now ready for use and the adaptor can be inserted.

Adaptor disassembly

To disassemble the adaptor on BPG 300 columns, refer to Figure A-3 and proceed as follows:

1 Locate the small slot at the base of the filter net ring. Remove the filter net (41/42) by twisting a spatula or similar stainless blunt blade in the slot. Remove the support net (40).
2 Remove the stop screw (28) and locking nut (27) from the top plate (26).
3 Release the sealing unit (30) and inner adaptor tube (35) by unscrewing the bolts (31) on the upper ring of the sealing unit.
4 Carefully remove the seal adjuster (23) releasing the tension on the spring (38) situated under the inner adaptor tube (35).
5 Remove the top-plate assembly (24 to 26, 29 and 32) and separate it from the outer adaptor tube (36) by turning it in a clockwise direction.
6 Remove the sealing unit (30) and the adaptor O-ring (33/34).
7 Remove the outer adaptor tube (36), the inner adaptor tube (35) and spring (38) from the adaptor plate (39) by removing the screws (37).
8 The top plate assembly (24-26, 29 and 32) can be disassembled by removing the screws (32).

This completes the adaptor disassembly procedure.

Adaptor assembly

To assemble the adaptor on BPG 300 columns, refer to Figure A-3 and proceed as follows:

1 Position the bushing rings (25) in the top plate (26).
2 Using the screws (32), secure the adjuster nut insert (29) to the adaptor height adjuster body (24) through the top plate (26) so that the adaptor height adjuster body turns on the bushing rings (25).
3 Slide the spring (38), inner adaptor tube (35) and outer adaptor tube (36) onto the adaptor plate (39).
4 Fasten the outer adaptor tube (36) to the adaptor plate (39) using the screws (37).
5 Slide the sealing unit (30) onto the outer adaptor tube (36).
6 In a counter clockwise direction, screw down the top plate assembly (24 to 26, 29 and 32) approximately 5 cm onto the outer adaptor tube (36).

7 Turn the seal adjuster (23) clockwise onto the top of the adaptor until the holes in the inner adaptor tube (35) are in line with the holes in the outer adaptor tube (36).

8 Finger-tighten the screws (31) into the upper half of the sealing unit (30), making sure they remain level and aligned correctly with the holes until 1-2 mm of the screw thread shows. Finally, tighten the screws with a ring spanner.

9 Locate the stop screw (28) and locking nut (27) in the top plate (26). Turn the stop screw until it engages the groove in the outer adaptor tube (36), preventing sideways movement of the tube without preventing vertical movement. Lock the stop screw (28) in position with the locking nut (27).

10 Mount the adaptor O-ring (33/34) on the adaptor plate (39).

11 Wet the filter net (41/42) with 20% ethanol to remove all air bubbles trapped in the filter net. If the filter net is tight, warm it in 40°C water.

12 Mount the distribution plate (13), support net (40) and the filter net (41/42) on the adaptor plate (39).

This completes the BPG 300 adaptor assembly procedure.

Mounting an extension tube (packing device)

The extension tube is assembled when delivered. Disassembly/ assembly of the extension tube is done the same way as with the column, with exception of the stand directives.

To mount an extension tube on the BPG 300 column, refer to Figure A-4 and proceed as follows:

1 Remove the adaptor from the column and place the extension tube onto the upper flange (5). Make sure that the thin O-ring is properly positioned in the flange.

2 Use the top plate clamp to tighten the extension tube to the column.

The extended column is now ready for the first packing step in two-step packing procedures. The extra clamp supplied with the extension tube is used to mount the adaptor on the extension.

Note: *Do not pack the column to bed heights higher than the length of the column, i.e. the adaptor should seal against the column wall, NOT against the extension tube wall. A tube length of 10 cm is required when installing the adaptor into the column tube if the packing extension is removed.*

4.1.3 BPG 450

Column disassembly

To disassemble the BPG 450 column, refer to Table A-3 and Figure A-5 at the back of this manual and proceed as follows:

1 Loosen the adaptor sealing by turning the handle (24).

WARNING! If the adaptor O-ring seal is dry, forced movement of the adaptor can damage it. Wet the O-ring with water or 20% ethanol solution. DO NOT grease the O-ring.

2 Move the adaptor down a few millimeters to loosen the O-ring from the glass wall. Then move it up as far as it will go.

3 Remove the bolts (26) and washers (27). Loosen the lid (17) and lift out the adaptor.

Note: *The adaptor on BPG 450 columns is heavy. For safety reasons, it is recommended that the adaptor is lifted with a ceiling hoist connected to the column lid eye bolts.*

4 Loosen the bolts (44) to separate the column tube (12) [including flanges (3)] from the end-piece (1). Carefully lift off the column tube. If the O-ring adheres to the glass tube or the end piece, wet the O-ring with 20% ethanol and then remove it.

5 To change the top and bottom flange O-rings, loosen the Allen screws (7) and carefully lift off the top flange. Remove the glass tube and replace the bottom O-ring. Put back the glass tube and fit the new top O-ring. Finally, fit the top flange. Be careful to align the glass tube with the flanges.

This completes the adaptor disassembly procedure.

Column assembly

To assemble the BPG 450 column, refer to Figure A-5 and Table A-3, and proceed as follows:

1 Referring to Figure 4-6 below, ensure that the following items are positioned correctly in the end piece:

- Distribution plate (41)
- Snap plug (36)
- Support net (2)
- Filter net (5)
- U-shaped seal (4; mounted on the filter net)

Note: *Wet the filter net with 20% ethanol to eliminate trapped air.*

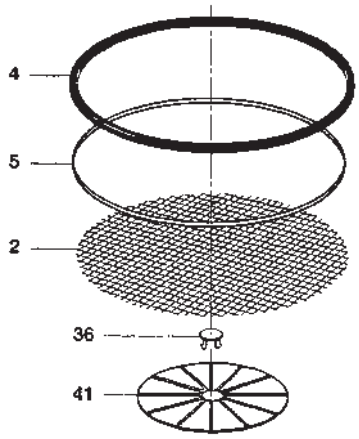

Fig 4-6. End-piece component location for BPG 450 columns.

2 Carefully place the column tube (12) on the U-shaped seal (4). Ensure that the flange (3) is centered on the U-shaped seal.

3 Secure the tube to the end-piece with the bolts (44). Tighten the bolts in cross-wise sequence with the torque wrench set on 8 Nm. Each bolt has to be tightened 3 times.

WARNING! The column tube will crack if too much pressure is exerted when tightening the bolts which secure the flange (3) and the rods (8) to the column tube. Always use the torque wrench set on 4 Nm when tightening these bolts. See Table 4-3 below for torque wrench settings on the BPG 450 column.

Table 4-3. Torque wrench settings for BPG 450 columns.

Item (Refer to Fig. A-5 for location)	Torque wrench setting
Lid and end-piece bolts (26 & 27)	8 Nm
Glass tube to flanges (3) and rods (8)	4 Nm

Adaptor disassembly [To replace the O-ring (32)]

To replace the O-ring on BPG 450 columns, refer to Table A-3 and Figure A-5 and proceed as follows (also see torque wrench settings in Table 4-3):

1 Remove the adaptor screw bolts (39).
2 Lift off the lid (17) and adaptor screw (29). Take care not to bend the inlet tube (31).
3 Remove the spring (34).
4 Remove the screws (33) holding the inlet tube.
5 Remove the old O-ring (32) and discard it.

This completes the adaptor disassembly.

Adaptor assembly

1 Place the new lubricated (20% ethanol) O-ring (32) between the inlet tube (31) and the adaptor (38). Tighten the Allen screws (33).

2 Slide the spring (34)over the inlet tube.

3 Carefully lower the adaptor screw (29) and lid (17) over the inlet tube.

4 Screw the bolts (39) into the adaptor and tighten them.

The adaptor is now reassembled.

Mounting an extension tube (packing device)

The packing device for BPG 450 is a stainless steel extension tube.

To mount the extension tube on a BPG 450 column, proceed as follows:

1 Remove the adaptor from the column and place the stainless steel extension tube onto the upper flange. Make sure that the O-ring is positioned properly in the flange.

2 Use the bolts delivered with the extension tube to secure the extension tube to the column.

The extended column is now ready for the first packing step in two-step packing procedures.

Note: *Remove the extension tube after the initial sedimentation step. Insert the adaptor and continue packing. A tube length of 15 cm is required when installing the adaptor into the column tube if the packing extension is removed.*

4.2 Connections

Several different connectors are available to connect BPG columns to different types of tubing and systems (see Chapter 3 in the BPG Columns Maintenance Guide).

The hygienic, sanitary clamp fittings on BPG columns permit cleaning-in-place. Other connectors are made of polypropylene, with good chemical resistance. The outer diameter on all BPG clamp fittings is 25 mm. A 51 mm clamp is used with the manometer kits and the safety valve.

4.3 Grounding the column

To avoid discharge from static electricity, the column can be properly grounded (earthed).

Note: *The equipment must be grounded when it is to be used in a potentially explosive atmosphere.*

For this purpose, a suitable grounding kit is available as an accessory and a grounding connection terminal is provided on the column stand as shown below.

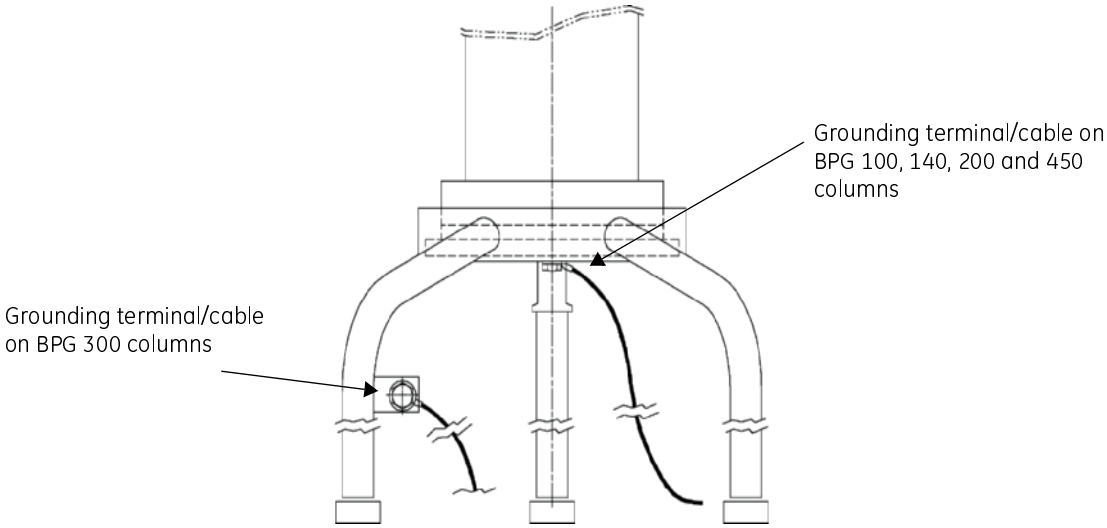

Fig 4-7. Grounding connection terminal on the column stand.

To ground the column, connect one end of the grounding cable to an earth terminal in the plant grounding network and the other end to the ground terminal located on the column stand. The column ground terminal has an M6 thread.

4.4 *Leakage test*

To test for leakage across the adaptor:

1 Fill the column with water or sanitizing solution and then position the adaptor in the column to the same height as you intend to run the column.

2 Seal the adaptor with the seal adjuster.

3 Remove the air in the column.

4 Close the column outlet valve.

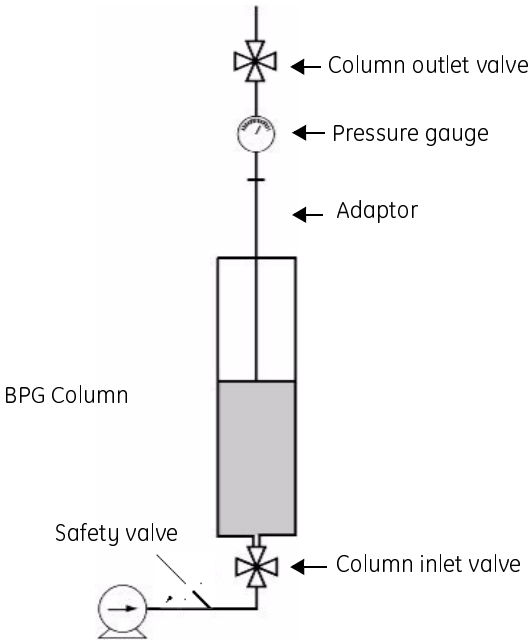

Fig 4-8. Schematic representation of the column, valves and pump for leakage testing.

5 Raise the pressure to maximum working pressure.

6 Wait about 15 minutes until the pressure has stabilized.

7 Check the pressure drop on the pressure gauge. The pressure drop should not exceed 0.1 bar during 5 minutes. A recorder linked to a pressure controller gives more precise figures.

This completes the leakage test.

5 Operation

WARNING! Please handle the column with care since the pressure specifications are only valid if the glass tube is undamaged and free from scratches. Never blow air or gas into the column.

Note: *Before packing the column for the first time we recommend that the column and tubing are flushed with CIP solution e.g. NaOH to ensure that all surfaces are clean. Refer to section 1.3 in the BPG Column Maintenance Guide.*

For optimum performance from BPG columns, we recommend the column packing protocols outlined in the paragraphs below. Before using the columns, it is recommended that you read the complete chapter.

5.1 Column packing

There are many different packing methods, each dependent on the column and type of media used. For BPG columns, we recommend the packing method described in sub-section 5.1.5. The optimum packing flow rate is dependent on temperature, type of media, batch and quantity. Consequently, for each individual system, the optimum flow rate must be determined empirically by producing a pressure/flow rate curve. The recommended methods are described comprehensively in sections 5.1.3 and 5.1.5.

5.1.1 Media quantities required per cm bed height

The media quantities required per cm bed height for BPG columns are given below.

Table 5-1. Media quantity requirements for BPG columns.

Media	Amount of media per liter of packed column	Amount of media per centimeter of packed bed				
		BPG 100	BPG 140	BPG 200	BPG 300	BPG 450
DEAE Sepharose Fast Flow	1.25 l	98 ml	192 ml	393 ml	882 ml	1.97 l
Sepharose Fast Flow	1.25 l	98 ml	192 ml	393 ml	882 ml	1.97 l
CM Sepharose Fast Flow	1.25 l	98 ml	192 ml	393 ml	882 ml	1.97 l
S Sepharose Fast Flow	1.25 l	98 ml	192 ml	393 ml	882 ml	1.97 l
Phenyl Sepharose Fast Flow	1.25 l	98 ml	192 ml	393 ml	882 ml	1.97 l
Sephadex G-25 C and M grades	250 g	19.6 g	38.2 g	78.5 g	176 g	394 g
Sephadex G-25 SF	280 g	22.0 g	42.9 g	88.0 g	198 g	443 g
Sepharose 4 and 6 Fast Flow	1.20 l	94 ml	183 ml	377 ml	847 ml	1.89 l
Sephacryl High Resolution	1.25 l	98 ml	192 ml	393 ml	882 ml	1.97 l
Superdex 75 and 200 prep grade	1.20 l	94 ml	183 ml	377 ml	847 ml	1.89 l
Q and SP Sepharose High Performance	1.20 l	94 ml	183 ml	377 ml	847 ml	1.89 l
Phenyl Sepharose High Performance	1.20 l	94 ml	183 ml	377 ml	847 ml	1.89 l

5.1.2 Recommended net sizes

It is important to select the appropriate adaptor and end-piece nets. Nets are available in 10, 12, 23 and 54 µm porosities. Improperly sized nets can lead to back pressure problems or media leakage. Recommended net sizes are as follows:

Table 5-2. Recommended net sizes for BPG column .

Net size (µm)	Material	Bead size (µm)
10	PA[1]	< 70
12	PEEK[2]	< 70
23	PP[3]	FF-media
54	PP[3]	BB-media

[1] Polyamide
[2] Polyetherether ketone
[3] Polypropylene

5.1.3 Optimum packing flow rate determination

To establish the optimum packing flow rate for BPG columns proceed as follows:

STEP 1

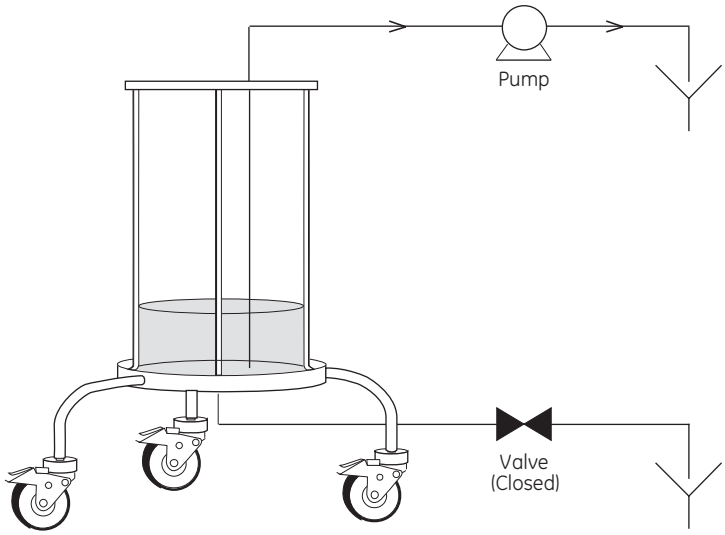

Fig 5-1. Optimum flow rate determination, step 1.

WARNING! Always try to keep hazardous fluids in closed containers to avoid gas or spillage in the working area.

STEP 1

a. Wet the column nets with 20% ethanol to eliminate trapped air.
b. Fill the column with 10-15 cm of packing buffer.
c. Drain the tubes to remove all air bubbles.
d. Connect a tube to the suction side of a pump.
e. Start the pump, place the tube onto the bottom net and extract any remaining air bubbles trapped under the net.

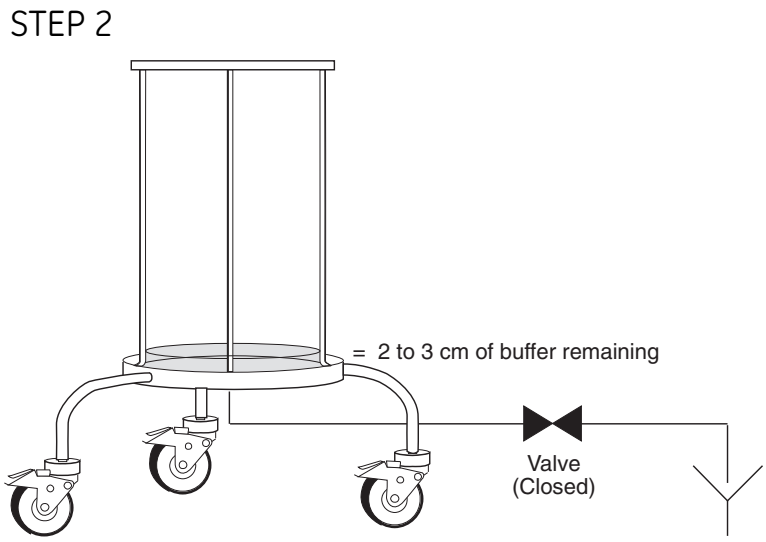

Fig 5-2. Optimum flow rate determination, step 2.

STEP 2

a. Continue to pump buffer (and air) until there is only 2-3 cm of buffer remaining in the column.

b. Use a spirit level to check that the column is standing horizontally. Correct with the adjustable feet/wheels as necessary.

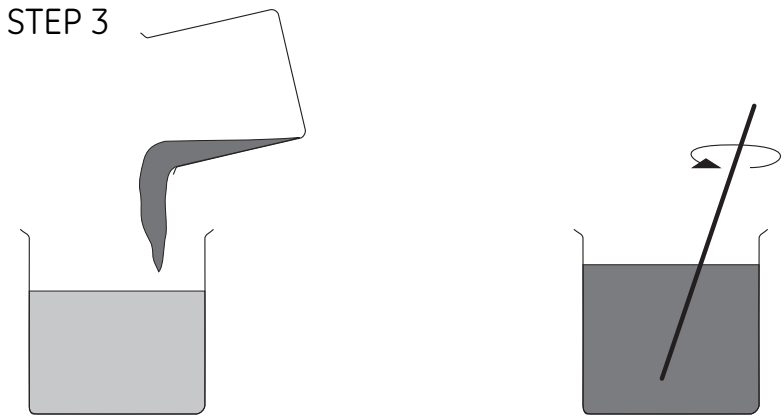

Fig 5-3. Optimum flow rate determination, step 3.

STEP 3

a. Mix the packing buffer with the media to form a 75% slurry i.e. packed volume/ slurry volume = 0.75.

Note: *This slurry volume is appropriate for BioProcess Media. For other chromatography media check recommended slurry volume with your vendor.*

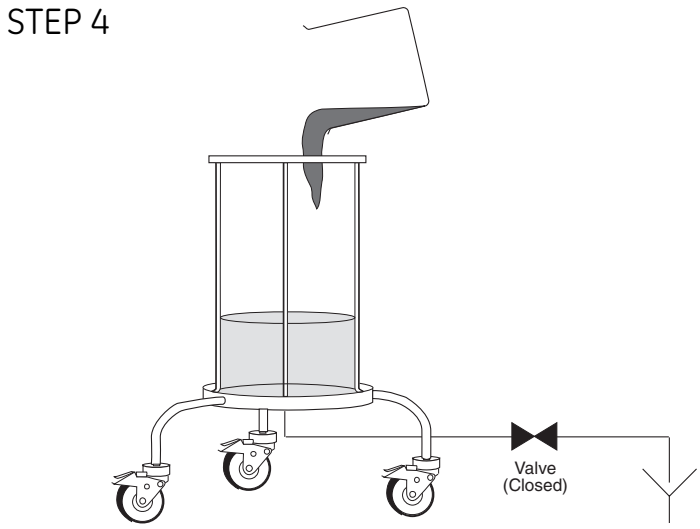

Fig 5-4. Optimum flow rate determination, step 4.

STEP 4

a. Carefully pour the homogenous slurry into the column. Make sure that no air bubbles are trapped within the slurry.

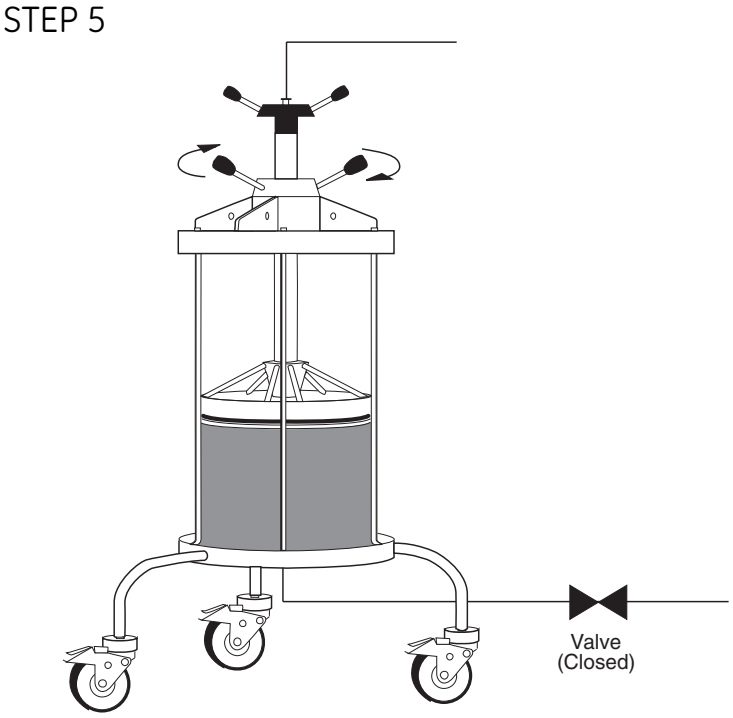

Fig 5-5. Optimum flow rate determination, step 5.

STEP 5

Note: *This step should be completed quickly so that the media does not sediment completely.*

a. Insert the adaptor.

b. Use the four bolts (BPG 100, 140 and 200) or top plate adaptor clamp (BPG 300 and 450) to secure the column adaptor in place.

c. Use the height adjuster handle to position the adaptor 1-5 mm below the top surface of the liquid.

STEP 6

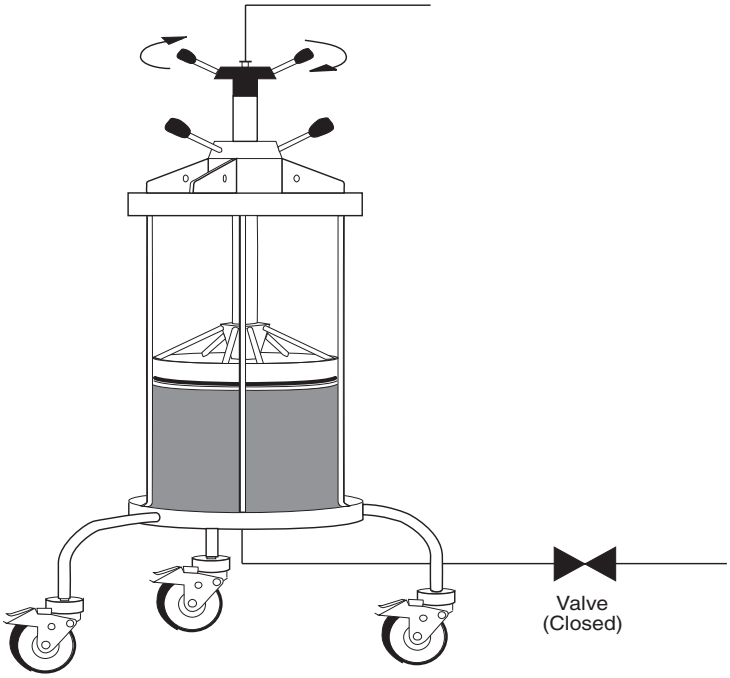

Fig 5-6. Optimum flow rate determination, step 6.

a. Seal the adaptor O-ring by turning the seal adjuster knob (BPG 100, 140 and 200), or handle (BPG 300 and 450) on the top of the adaptor, clockwise.

b. With the O-ring sealed, lower the adaptor, filling it with buffer to expel any air in the adaptor tube. The adaptor tube is full when the buffer creates a meniscus and no further air bubbles appear.

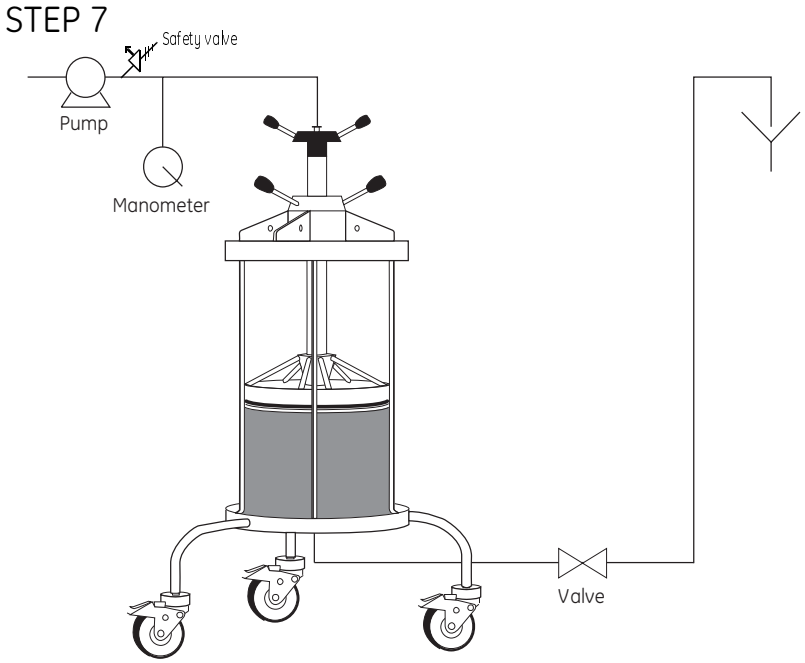

Fig 5-7. Optimum flow rate determination, step 7.

STEP 7

WARNING! Always use a safety valve connected directly after the pump.

a. Start the pump and remove air in the tubing from the pump by pumping with a low flow rate.

b. While pumping at the low flow rate, connect a manometer between the pump and the column, and then connect the tubing to the top of the column.

c. Open the bottom valve.

d. Establish a pressure/flow rate curve by beginning with a low flow rate. The media will begin to settle, leaving a clear space between the bed and the adaptor. Do NOT adjust the adaptor.

e. Slowly increase the pressure in increments and record the flow rate when the pressure has stabilized. The time taken for the pressure to stabilize is dependent on the type of media, it can take from a few minutes to a few hours.

f. Continue to increase the pressure until a flow rate plateau is reached (increased pressure does not give higher flow rates). For some rigid gel media, there will be no plateau. In those cases, use the flow rate at maximum allowable pressure for the column.

g. When the maximum pressure has been reached, the determination should be concluded.

STEP 8

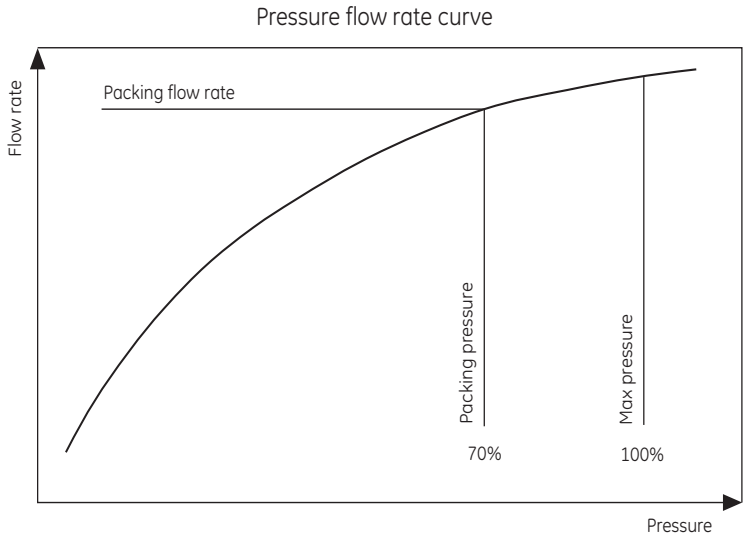

Fig 5-8. Optimum flow rate determination, step 8.

STEP 8

a. Plot the pressure against the flow rate, as indicated in the above example. The optimal packing flow rate is 70–100% of the maximum flow rate. For some very rigid media there will be no plateau, in those cases use the flow rate at maximum allowable pressure for the column.

5.1.4 Recommended packing flow rates for Amersham Biosciences media

Recommended packing flow rates will be found in the instruction accompanying each delivery of media. However, for optimal packing we recommend a pressure/flow curve determination be performed for each lot of media, column or system configuration as described in section 5.1.3.

5.1.5 Column packing method

This packing method can be used with BioProcess Media and is based on a constant flow rate being applied through the column. To prepare for the column packing procedure, proceed as follows:

WARNING! Always use a safety valve connected directly after the pump.

STEP 1. Confirm that the optimum flow rate for the combination of equipment, column, type of media and quantity has been established (refer to section 5.1.3).

STEP 2. Check that there is sufficient packing buffer available. A typical packing buffer is 0.1 M NaCl. However, always check the media instructions for packing buffers.

STEP 3. Wet the nets with 20% ethanol to eliminate trapped air in the nets.

STEP 4. Adjust the pump flow rate to the packing flow rate, without connecting the column to the pump. Record the pump speed in order to start the packing at the correct flow rate.

STEP 5. If the media is already in the column, lift up the adaptor, carefully stir the media and repeat steps 5 and 6 in section 5.1.3. If the media is not in the column, repeat steps 1–6 of section 5.1.3.

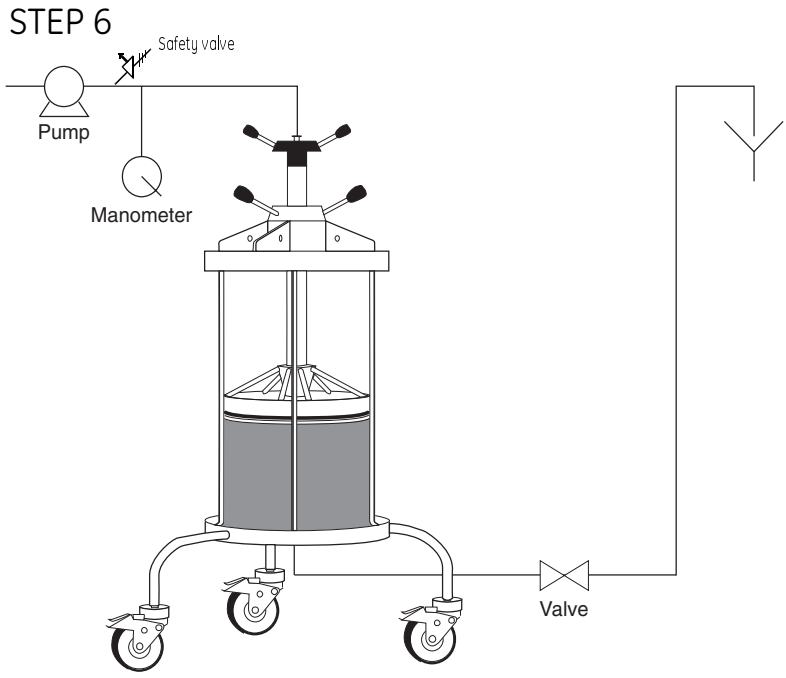

Fig 5-9. Column packing method, step 6.

STEP 6

a. Start the pump.
b. Remove air bubbles in the tubing from the pump by pumping with a low flow rate.
c. While pumping at the low flow rate, connect the manometer between the pump and the column, then connect the tubing to the top of the column.
d. Open the bottom valve.
e. Adjust the flow rate to the predefined rate.
f. Measure the flow rate at regular intervals and adjust if necessary. The back-pressure of the bed will increase and reduce the flow rate as the media packs.
g. Ensure that the pressure, displayed on the manometer, does not exceed the maximum pressure of the media (refer to user manual for each media) or the column.

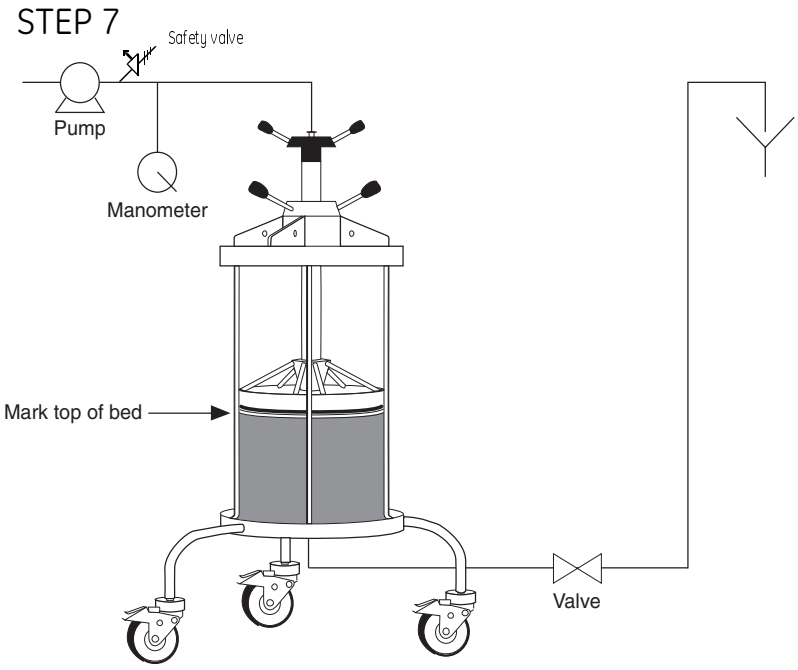

Fig 5-10. Column packing method, step 7.

STEP 7

a. When the bed has settled, mark the top of the bed.
b. Stop the pump.
c. Close the bottom valve.
d. Loosen the adaptor O-ring seal by turning the adjuster knob (BPG 100, 140 and 200), or the seal adjuster handle (BPG 300 and 450), on top of the column counter-clockwise.

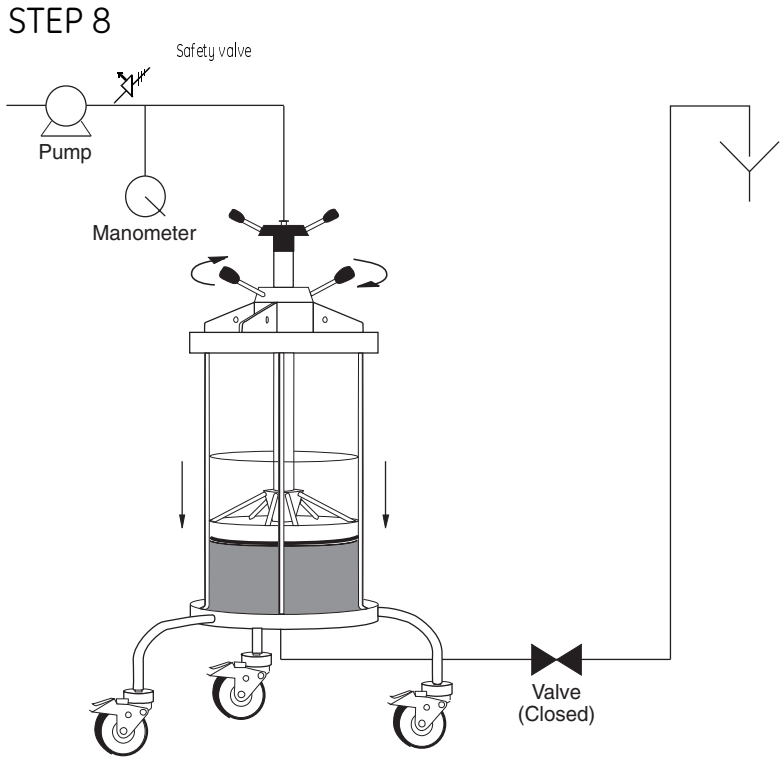

Fig 5-11. Column packing method, step 8.

STEP 8

a. Lower the adaptor quickly by turning the height adjuster handle clockwise. The buffer between the sedimented bed and the adaptor passes between the adaptor O-ring and the column wall. During this operation the bed will start to rise. Stop lowering the adaptor when it is 0.5 to 1 cm above the bed.

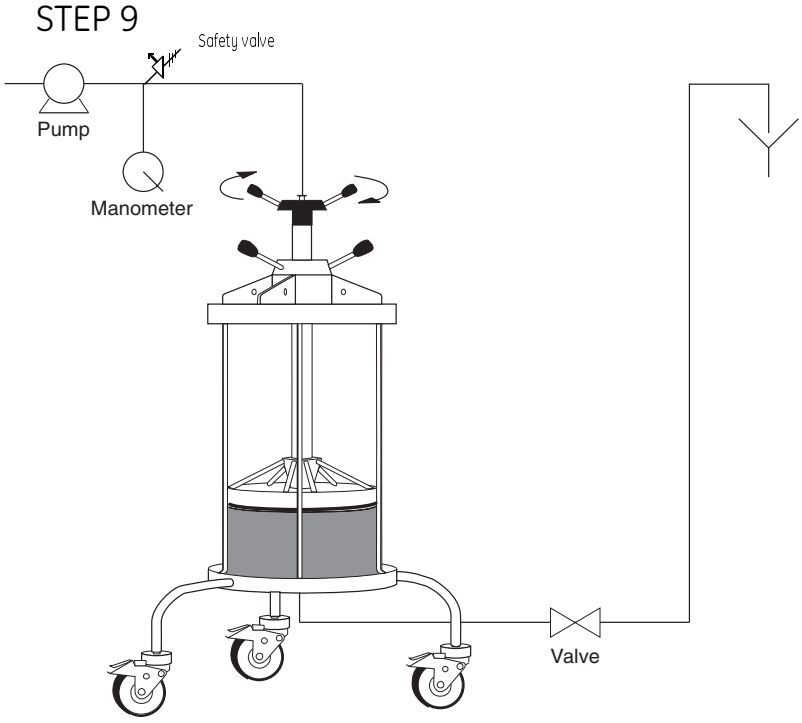

Fig 5-12. Column packing method, step 9.

STEP 9

a. Seal the adaptor O-ring by turning the knob (BPG 100, 140 and 200), or the seal adjuster handle (BPG 300 and 450), clockwise.

b. Open the bottom valve.

c. Start the pump.

d. Adjust the flow rate to the packing flow rate. Check the manometer to ensure that the pressure does not exceed the maximum pressure for the media (refer to user manual for each media). The bed will be compressed again to the level of the mark on the column (made in step 7).

e. Repeat steps 7 and 8 until the adaptor reaches the mark on the column.

Note: *When there is a maximum of 1 cm of liquid remaining, do not open the adaptor O-ring. Instead, use the height adjuster handle to screw the adaptor down the last remaining centimeter. Never lower the adaptor more than a maximum of 5 mm into the bed. Disconnect the tubing on the top of the column so that excess buffer can be removed via the top of the adaptor.*

This concludes the column packing procedure. Wash out any remaining buffer or slurry from the top of the adaptor. The space between the adaptor head and the top of the column should be dry and clean or filled with 20% ethanol in order to prevent bacterial growth.

Note: *Before using the column, always check the performance of the packed column. See instructions for this procedure in section 5.2 below.*

5.2 Column evaluation

The efficiency of a column depends on how well it is packed. A poorly packed column gives rise to uneven flow, resulting in zone broadening and reduced resolution. It is thus important to have a method by which the column can be tested before it is put into operation. Such a method should be simple, quantitative and should not introduce contaminating materials. It is also an advantage if the same method can be used to monitor column performance over its working life, so that it is easy to determine when the media should be re-packed or replaced.

Methods that use colored compounds, such as Blue Dextran, should be avoided since they do not meet the above criteria and cannot be used with ion exchange and affinity chromatography media.

Experience has shown that the best method of expressing the efficiency of a column is in terms of the height equivalent to a theoretical plate, HETP, and the peak asymmetry factor A_s. These values can be determined easily by applying a sample of a NaCl or acetone solution, for example, to the column.

It is important that the column is properly equilibrated before the packing is evaluated (2 column volumes). It is preferable to run three test runs to see whether the test values are stable. If a poor result improves during this test, the reason can be that the column was not properly equilibrated. To check that the bed is stable, run the column at 70% packing pressure for 20 hours and test it again (preferably three test runs).

Note that pressure spikes may cause poor packing (cracking). If this happens, an air trap and a pressure relief valve are needed between the pump and the column. The pressure relief valve should be placed between the air trap and column.

5.2.1 Choice of test sample for columns

The most appropriate material for column testing is of course the sample that is to be run in the application, but this is not always practical or economical. As an alternative, a solution of either NaCl or acetone can be used to give a good indication of the column packing quality. The eluate is monitored by measuring

the conductivity or UV absorption, the resulting elution profile is used to calculate the HETP value.

The advantages of using a NaCl solution are that it is readily available and can be used safely to test all columns. One disadvantage is that NaCl may interact with the media matrix, especially ion exchanger matrices, and thus give erroneous results.

Acetone, however, does not interact with the matrix and is detected by UV absorption at 280 nm. Alternatively, the running buffer concentration can be increased 10-fold and used as a test solution.

Fig. 5-13 below shows a UV trace for acetone in a typical BPG column application and gives the calculated HETP and A_s values.

Column: BPG 300
Media: Sepharose 6 Fast Flow
Bed height: 57.5 cm
Bed volume: 40.6 liters
Eluent: Distilled water
Sample: 1.05 liters
(1% acetone, 1%NaCl)
Flow rate: 19 cm/h
V_e = 18.8
W_h = 0.9
HETP = 0.024 cm
a = 0.90
b = 0.85
b/a = 0.85/0.90
A_s = 0.94

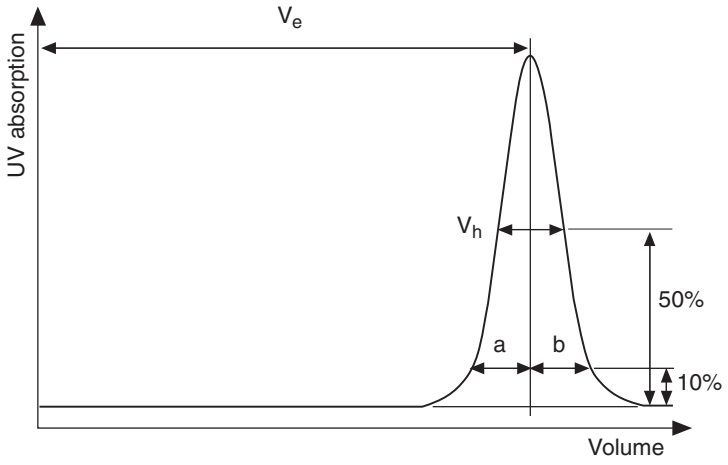

Fig 5-13. A test chromatogram showing HETP and A_s calculations.

5.2.2 HETP calculation

The sample volume should be approximately 1% of the total bed volume and the concentration 1.0% v/v NaCl, or equivalent when using stronger buffer. Alternatively, 1.0% v/v acetone can be used. The linear flow rate should be 30 cm/h for 34 µm and 50 µm media and 20 cm/h for 90 µm media. To avoid dilution of the sample, apply the sample as close to the column inlet as possible. If an airtrap is included in the system, it should be by-passed during sample application to avoid back-mixing. Calculate the HETP value from the conductivity (or UV) curve as follows:

HETP, in its simplest terms, is expressed as:

HETP = L/N

where,

L = Bed height (cm)

N = Number of theoretical plates.

N is defined by the equation:

$\mathbf{N} = 5.54 (V_e / W_h)^2$

where,

$\mathbf{V_e}$ = Elution volume (ml)

$\mathbf{W_h}$ = Peak width at half height (ml)

$\mathbf{V_e}$ is measured as the volume passed through the column from the middle of the sample application to the peak maximum.

$\mathbf{W_h}$ is measured as the width at the half-height of the peak (from the base to the top).

From the example in Fig. 5-13, the HETP value can be calculated from the chromatogram as follows:

Table 5-3. HETP calculations.

	V_e (ml)	W_h (ml)	N	N/m	HETP cm
Acetone	18800	900	2417	4203	0.024

Well packed columns will have low HETP values. It is however only possible to compare columns that have been packed with the same type of media and that have been tested under identical conditions.

As a general rule-of-thumb, a good HETP value is approximately two to four times the mean bead diameter of the media in question, provided that the sample is not interacting with the media.

In practice, the correlation between the HETP value and the column performance can only be assessed by the column operator. Once this has been established, a standard can be set to judge the acceptability of a column packing.

For example, the column operator may know from experience that a column packed with Sephadex G-25 Media with HETP values above 0.05 cm does not give the required separation. Consequently, the column operator will set this value to the maximum permissible i.e. the minimum acceptable quality.

5.2.3 Peak asymmetry factor calculation

The shape of the peak should be as symmetrical as possible. This is usually the case for gel filtration media, but for certain ion exchange and affinity media, it may be asymmetrical due to interaction with the media.

A change in the shape of the peak is usually the first indication of column deterioration. The peak asymmetry factor should be as close as possible to 1.

The peak asymmetry factor, $\mathbf{A_s}$, is calculated from the graph in Fig. 5-13 as follows:

$$\mathbf{A_s = b/a}$$

where,

a = distance from peak apex to 10% of the peak height on the ascending side of the peak

b = distance from peak apex to 10% of the peak height on the descending part of the peak

Note: *Measuring the HETP and A_s values is the best way to judge the condition of the packed column. A packed column can look good, but still need repacking for optimal performance. Always check the column after packing and regularly between runs to ensure best column performance.*

6 Abbreviations

The abbreviations used in this manual are defined as follows:

A_s	Peak asymmetry factor
CIP	Cleaning-in-place
EPDM	Ethylene propylene
FEP	Fluroethenepropene
FF	Fast Flow
HETP	Height equivalent to a theoretical plate
HR	High resolution
ID	Internal diameter
IEX	Ion exchange chromatography
L	Bed height
N	Number of theoretical plates
PA	Polyamide
PEEK	Polyetheretherketone
POM	Acetal plastic
PP	Polypropylene
PTFE	Polytetrafluoroethane
PVC	Polyvinyl chloride
THF	Tetrahydrofurane
UV	Ultra violet
V_e	Elution volume
W_h	Peak width at half peak height
PFR	Per fluor rubber
FPM	Fluor carbon rubber

Table A-1: Components of BPG 100, 140 and 200 columns. Item numbers refer to Figures A-1 and A-2.

Item no.	Description	Material	In contact with process liquid?	Qty/ pk	BPG 100 Code no.	BPG 140 Code no.	BPG 200 Code no.
1	Column tube 500 Column tube 750 Column tube 950	Borosilicate glass Borosilicate glass Borosilicate glass	X X X	1 1 1	18-0251-01 18-0251-02 18-0251-03	18-1112-95 - 18-1112-96	18-1152-01 18-1152-02 18-1152-03
2	Rod for tube 500 Rod for tube 750 Rod for tube 950	316 316 316		1 1 1	18-1105-80 18-1105-81 18-1105-82	18-1105-83 - 18-1105-85	18-1105-83 18-1105-84 18-1105-85
3	Guide ring	PTFE		1	18-0251-22	18-1112-97	18-0252-22
4	Net, 54 µm, end-piece Net, 23 µm, end-piece Net, 10 µm, end-piece Net, 12 µm, end-piece	PP PP PA PEEK	X X X X	2 2 2 2	18-1126-97 18-9252-01 18-0251-77 18-1148-38	18-1126-99 18-1113-00 18-1113-02 18-1148-40	18-1127-01 18-9254-01 18-0252-77 18-1148-42
5	Support net, end-piece	PP	X	2	18-0251-55	18-1112-98	18-0252-55
6	Flange O-ring	EPDM	X	2	18-8494-01	18-1113-06	18-8489-01
7	Flange O-ring	FEP	X	1	18-0019-41	18-1113-07	18-0019-51
8	Nut, M6 Nut, M8	316 316		5 5	19-1582-01 -	- 19-0763-01	- 19-0763-01
9	Cup spring washer	316		4	18-8482-01	18-8482-01	18-8482-01
10	Flange	316		1	18-1134-21	18-1113-21	18-1134-22
11	Column cantering dowel	PTFE		8	18-1134-21	-	18-1134-22
12	End-piece	316L	X	1	18-1144-18	18-1113-20	18-1112-62
13	Bolt, M8 x 12	316		4	-	18-1113-28	18-1113-28
14	Washer	316		4	18-1143-26	18-1113-29	18-1113-29
15	Stand, complete	316L		1	18-1031-10	18-1031-20	18-1031-20
16	Clamp, 25 mm	304		1	18-1001-31	18-1001-31	18-1001-31
17	Adjusting knob	PEEK		1	18-1109-27	18-1109-27	18-1109-27
18	Domed nut	316		4	18-1143-25	18-1113-27	18-1113-27
19	Adjuster nut, body	316		1	11-0003-01	18-1113-26	18-1113-26
20	Bushing ring	POM		2	18-0264-01	18-0267-01	18-0267-01
21	Top plate	316		1	18-1143-24	18-1113-24	-
22	Adjuster nut, insert	PEEK		1	18-0251-20	18-0252-20	18-0252-20
23	Allen screw, M8 x 45	316		5	19-6375-01	19-6375-01	19-6375-01
24	Allen screw, M4 x 14	316		5	19-6356-01	19-6356-01	19-6356-01
25	Stopper	316		1	-	18-1113-25	18-1113-25
26	Adaptor head screw	316		3	18-8457-01	18-8457-01	18-8457-01
27	Sealing unit	316L		1	18-1147-47	18-1113-31	18-1147-48
28	Adaptor O-ring	EPDM	X	2	18-8475-01	18-1113-10	18-0275-01
29	Adaptor O-ring	FEP	X	1	18-0019-40	18-1113-11	18-0019-50
30	Inner adaptor tube	316		1	18-1113-23	18-1113-23	18-1113-23
31	Outer adaptor tube	316		1	18-1134-83	18-1134-83	18-1134-83
32	Screw, M6 x 12	316L/PTFE		3	18-1145-08	18-1145-08	18-1145-08
33	Spring	316/PTFE		1	18-8491-01	18-8491-01	18-8491-01
34	Adaptor plate**	S31803***	X	1	18-1112-80	18-1113-30	18-1112-81
35	Support net, adaptor	PP	X	2	18-1103-04	18-1112-99	18-0252-56
36	Net, 54 µm adaptor Net, 23 µm adaptor Net, 10 µm adaptor Net, 12 µm adaptor	PP PP PA PEEK	X X X X	2 2 2 2	18-1126-96 18-1103-08 18-1103-05 18-1148-37	18-1126-98 18-1113-01 18-1113-03 18-1148-39	18-1127-00 18-9253-01 18-0252-76 18-1148-41
37	Gasket 25 mm	EPDM	X	2	18-0019-27	18-0019-27	18-0019-27
38	Feet, stand	316		1	18-1126-93	18-1126-93	18-1126-93
39	Wheel	-		3	18-1001-09	18-1001-09	18-1001-09
40	4-port, 2-way valve	316L/PTFE	X	1	18-5757-01	18-5757-01	18-5757-01
41	Tubing*	-	X	1	-	-	-
42	Washer	PP		1	18-1142-79	NA	NA
43	Washer	PP		1	18-1142-80	18-1142-80	18-1142-80

* See Table 3-3 in the BPG Maintenance Guide; ** Tube i.d. 4 mm (BPG 100), 6 mm (BPG 140 and 200); *** Comparable to 316L

Table A-2: Components of BPG 300 columns. Item numbers refer to Figures A-3 and A-4.

Item no.	Description	Code number	Material	In contact with process liquid?	Qty/ pk
1	Adaptor top plate	18-1012-48	316		1
2	Nut, M12	18-1012-24	316		6
3	Cup spring washer	18-1012-25	316		6
4	Nut, M10	18-1012-44	316		5
5	Flange		316		
6	O-ring, 301 x 6 mm	18-1012-26	EPDM	X	2
7	O-ring, 301 x 6 mm	18-1012-27	FEP	X	1
8	Rod for T300/500 Rod for T300/750 Rod for T300/950	18-1012-31 18-1012-32 18-1012-33	316 316 316		1 1 1
9	Column tube T300/500 Column tube T300/750 Column tube T300/950	18-1012-28 18-1012-29 18-1012-30	Borosilicate glass Borosilicate glass Borosilicate glass	X X X	1 1 1
10	Net, 23 µm, endpiece	18-1012-34	PP	X	2
11	Net, 10 µm, endpiece Net, 12 µm, endpiece Net, 54 µm, endpiece	18-1012-35 18-1148-44 18-1127-03	PA PEEK PP	X X X	2 2 2
12	Support net, endpiece	18-1012-36	PP	X	2
13	Distribution plate	18-1012-37	PP	X	2
14	Endpiece	18-1140-47	316L	X	
15	Gasket, 25 mm, i.d. 10 mm	18-0200-00	EPDM	X	5
16	Gasket, 25 mm, i.d. 10 mm	18-1012-40	PTFE	X	5
17	Connector 25 mm/10 mm*	-	PP	X	2
18	4-port, 2-way manual valve	18-1012-56	316L/PTFE	X	1
19	Stand, complete	18-1012-38	316L		-
20	Washer	18-1012-61	316		6
21	Bolt, M12 x 25	18-1012-65	316		6
22	Wheel	18-1001-09	-		1
23	Seal adjuster	18-1012-41	PEEK		1
24	Adaptor height adjuster	18-1128-31	316		1
25	Bushing ring	18-1012-42	POM		2
26	Adaptor top plate	18-1129-06	316L		-
27	Nut, M10	18-1012-44	316		5
28	Stop screw, complete	18-1012-43	316/POM		1
29	Adjuster nut, insert	18-1012-45	PEEK		1
30	Sealing unit	18-1147-50	316L		-
31	Adaptor head screw, M8	18-1012-46	316		3
32	Allen screw, M8 x 60	18-1012-47	316		5
33	O-ring, 274 x 8 mm	18-1012-51	EPDM	X	2
34	O-ring, 274 x 8 mm	18-1012-52	FEP	X	1
35	Inner adaptor tube	18-1147-83	316		
36	Outer adaptor tube	18-1142-93	316L		1
37	Screw, M8 x 16	18-1145-10	316L/PTFE		5
38	Spring	18-1012-50	316/PTFE		1
39	Adaptor plate**	18-1112-82	S31803***	X	1
40	Support net, adaptor	18-1012-53	PP	X	2
41	Net, 23 µm, adaptor	18-1012-54	PP	X	2
42	Net, 10 µm, adaptor Net, 12 µm, adaptor Net, 54 µm, adaptor	18-1012-55 18-1148-43 18-1127-02	PA PEEK PP	X X X	2 2 2
43	Tubing*	-	PVC	X	
44	Clamp, 25 mm	18-1001-31	304		1
45	Wheel support	18-1033-33	POM/316		1

* See Table 3-4 in the BPG Maintenace Guide; ** Tube i.d. 10 mm; *** Comparable to 316L

Table A-3: Components of BPG 450 columns. Item numbers refer to Figure A-5.

Item no.	Description	Code number	Material	In contact with process liquid?	Qty/ pk
1	Endpiece	18-1109-67	316L	X	1
2	Support net, endpiece	18-1104-35	PP	X	1
3	Flange	18-1109-68	PP/316	X	1
4	U-shaped seal	18-1104-40	EPDM	X	1
	U-shaped seal	18-1117-55	PFR	X	1
5	Net, 54 µm, endpiece	18-1127-05	PP	X	1
	Net, 23 µm, endpiece	18-1103-19	PP	X	1
	Net, 10 µm, endpiece	18-1103-18	PA	X	1
	Net, 12 µm, endpiece	18-1148-46	PEEK	X	1
6	Flange O-ring 446 x 6	18-1105-33	EPDM	X	2
	Flange O-ring 446 x 6	18-1117-67	FEP	X	1
7	Allen screw M8 x 50	18-1109-40	316		5
8	Rod BPG 450/500	18-1106-18	316		1
	Rod BPG 450/750	18-1103-09	316		1
	Rod BPG 450/1000	18-1103-10	316		1
9	Sealing unit	18-1109-69	316L		1
10	Pin bolt	18-3001-07	316		3
11	Stop screw	19-4363-01	316		2
12	Glass tube BPG 450/500	18-1103-14	Borosilicate glass	X	1
	Glass tube 450/750	18-1103-15	Borosilicate glass	X	1
	Glass tube 450/1000	18-1103-16	Borosilicate glass	X	1
13	Revolving stop cap	18-1107-43	POM		1
14	Revolving stop	18-1109-41	316L		1
15	Screw M8 x 50	18-1109-40	316		5
16	Spacer	18-1109-42	316L		1
17	Lid	18-1109-70	316		1
18	Bushing ring	18-1109-43	PP		2
19	Adaptor height adjuster	18-1109-44	POM		1
20	Handle	18-1109-45	316		1
21	Nut flange	18-1109-46	PEEK		1
23	Seal adjuster	18-1109-47	PEEK		1
24	Handle	18-1109-48	316		1
25	Knob	18-1109-49	PP		1
26	Screw M10 x 65	18-1109-50	316		2
27	Washer 10.5 x 20	18-1109-51	316		10
28	Domed nut M10 and washer	18-1103-20	316		1
29	Adaptor screw	18-1109-54	316L		1
30	Adjustment tube	18-1109-55	316		1
31	Inlet tube i.d. 12 mm	18-1109-53	316L	X	1
32	O-ring 12.3 x 2.4	18-1103-70	EPDM	X	2
	O-ring 12.3 x 2.4	18-1117-65	FEP	X	1
33	Screw M4 x 16	18-1109-56	316		2
34	Spring	18-1107-44	316/PTFE		1
35	Screw M5 x 10	18-1109-57	316		2
36	Snap plug	18-1107-45	PP	X	2
38	Adaptor plate	18-1109-71	S31803*	X	1
39	Screw M8 x 25	18-1145-12	316L/PTFE		3
40	O-ring 419 x 10	18-1017-47	EPDM	X	1
	O-ring 419 x 10	18-1117-66	FEP	X	1
41	Distributor plate	18-1106-04	PP	X	2

*Comparable to 316L.

(continued on next page)

Components of BPG 450 columns (continued)

Item no.	Description	Code number	Material	In contact with process liquid?	Qty/ pk
42	Adaptor support net	18-1104-34	PP	X	1
43	Net 54 µm adaptor Net 23 µm adaptor Net 10 µm adaptor Net 12 µm adaptor	18-1127-04 18-1001-62 18-1017-46 18-1148-45	PP PP PA PEEK	X X X X	1 1 1 1
44	Screw M10 x 80	18-1109-59	316		2
45	Screw M10 x 16	18-1109-60	316		2
46	Bracket	18-1109-61	316		1
47	Screw M5 x 16	18-1109-62	316		2
48	Bottom inlet tube	18-1109-63	316L	X	1
50	Allen screw M10 x 40	18-1109-65	316		2
51	Leg	18-1109-66	316		1
52	Wheel support	18-1107-46	POM/316		1
53	Wheel Eye bolt	18-1177-75 18-1109-64			1 1
54	Adaptor stopper	18-1115-18	316		1

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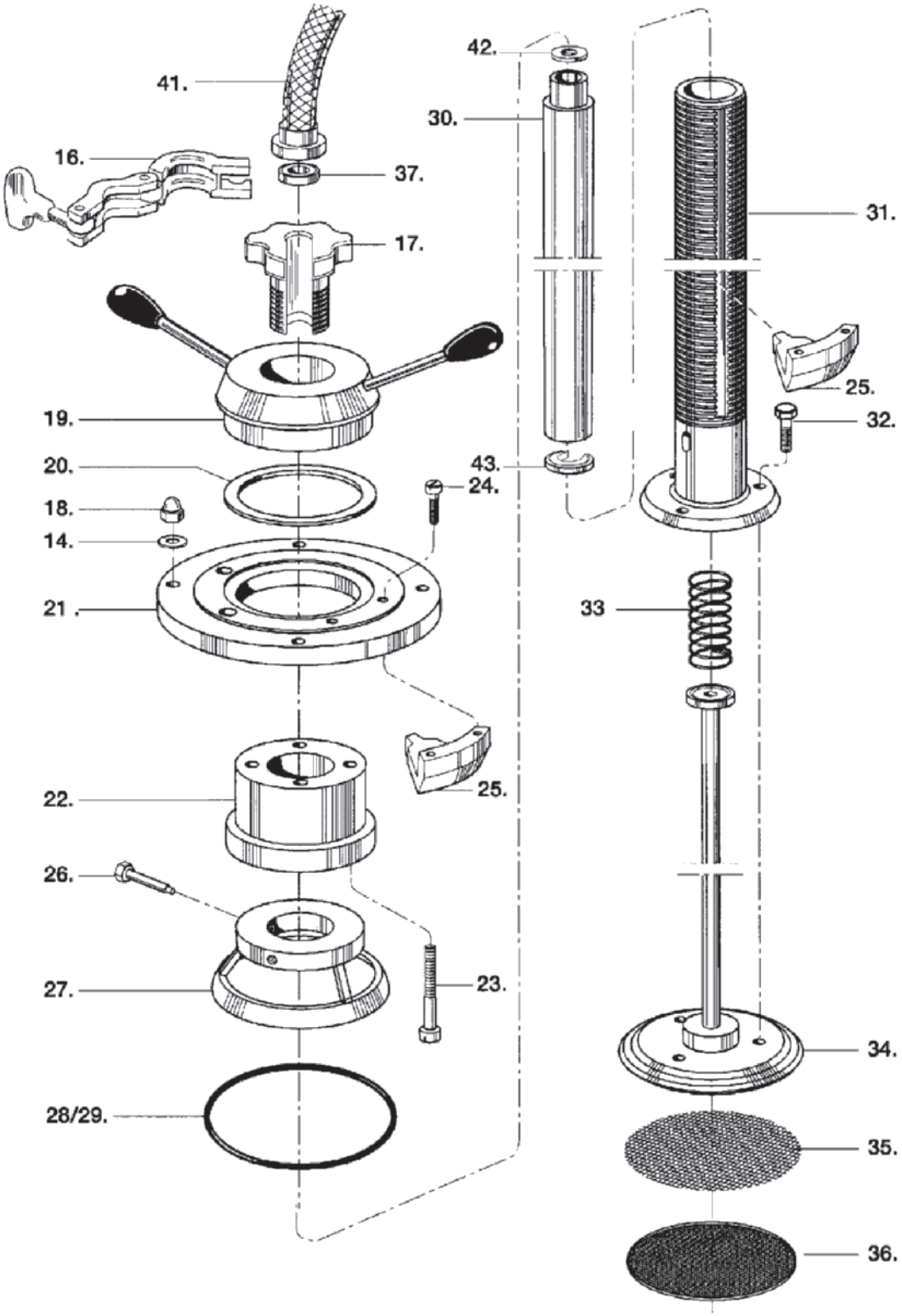

Figure A-1. BPG 100, 140 and 200 column parts identification.

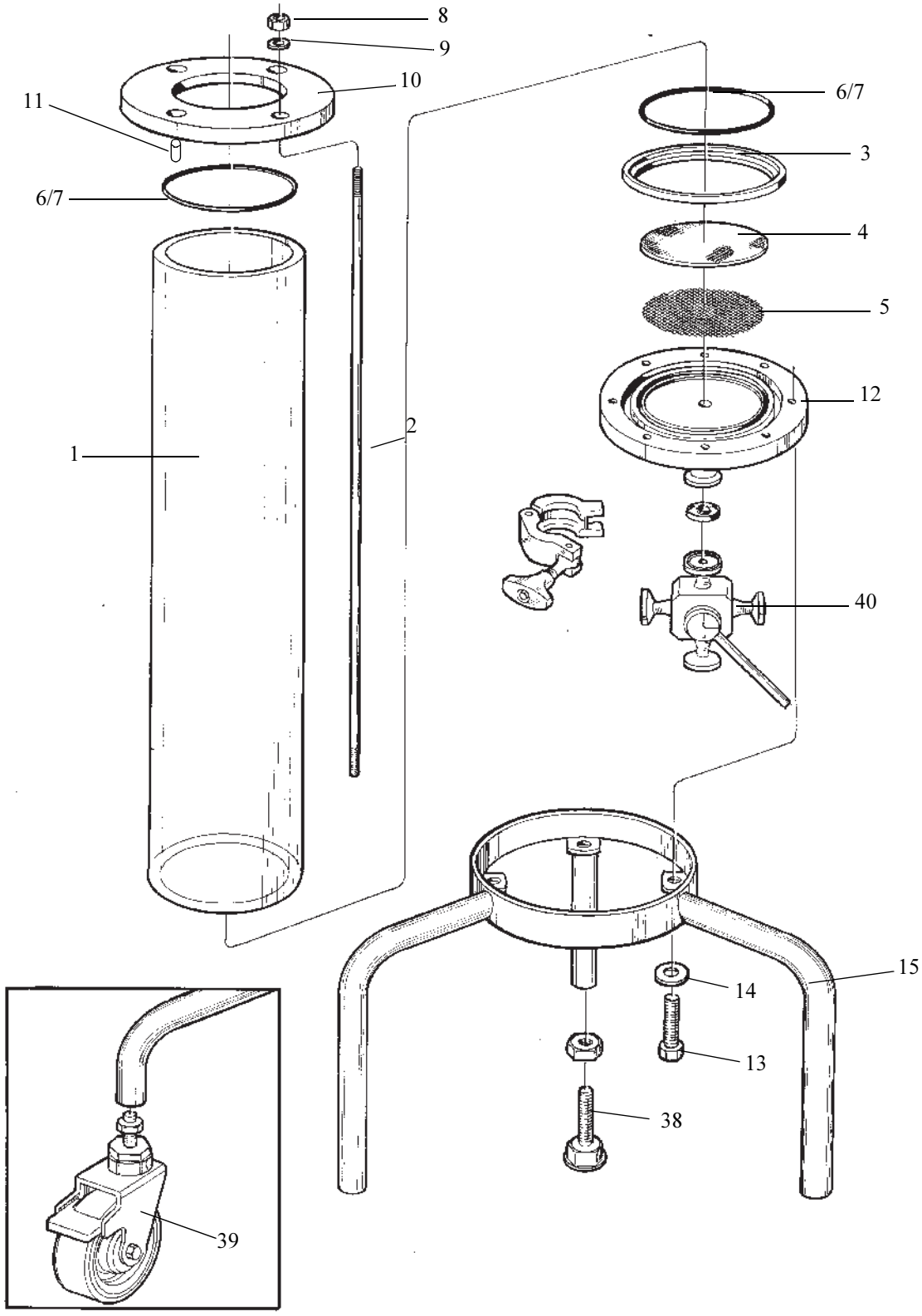

Figure A-2. BPG 100, 140 and 200 column parts identification (continued).

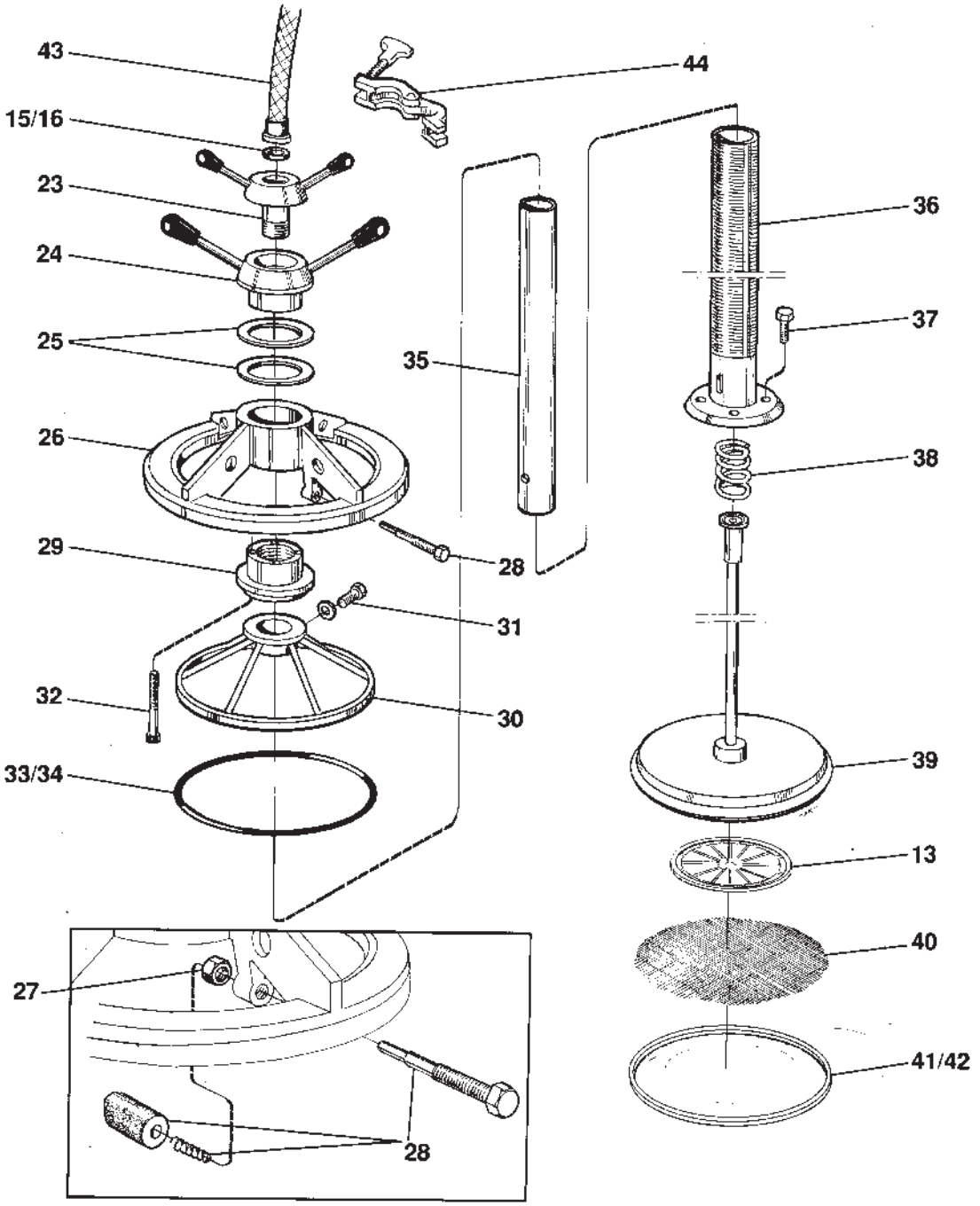

Figure A-3. BPG 300 column parts identification.

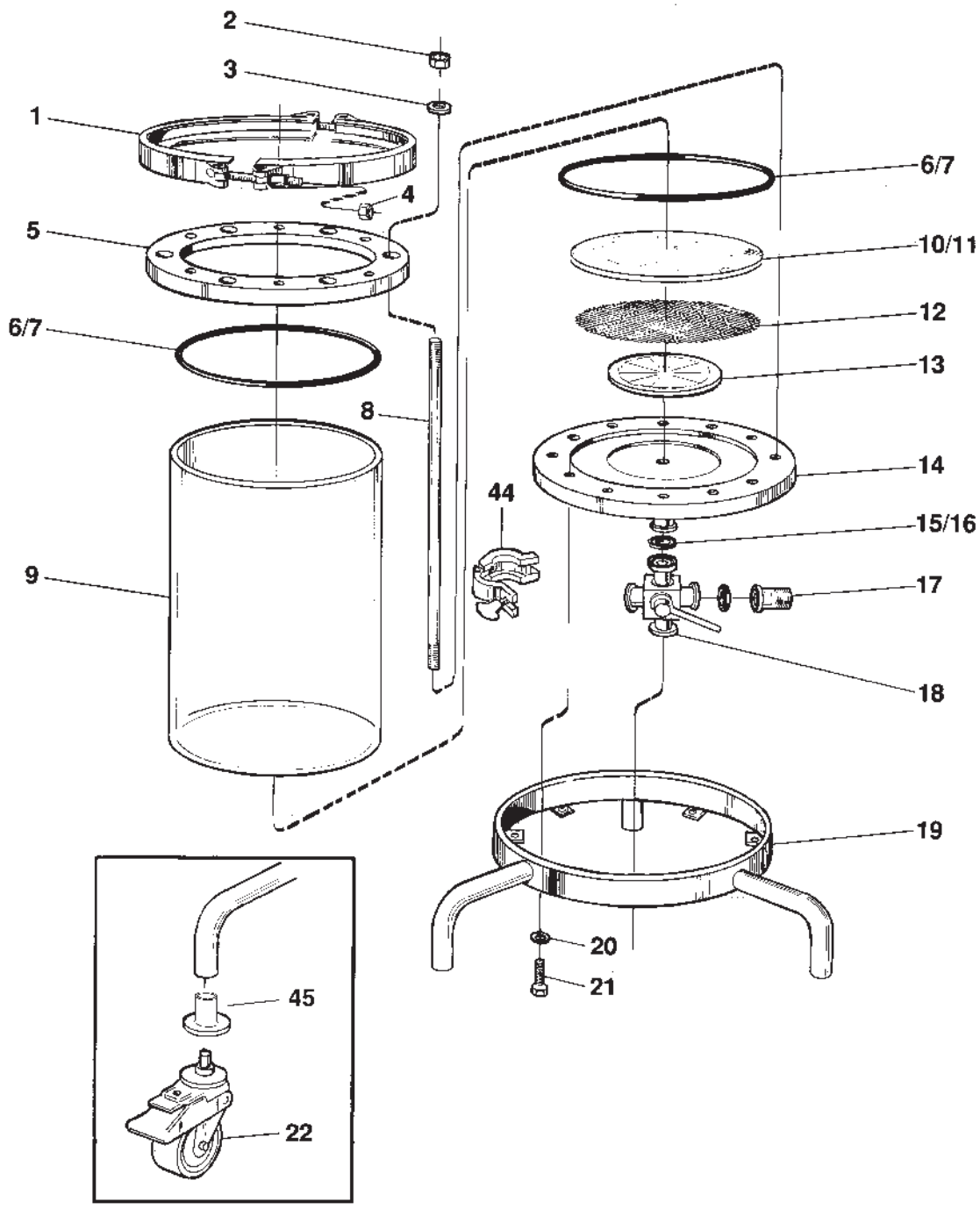

Figure A-4. BPG 300 column parts identification (continued).

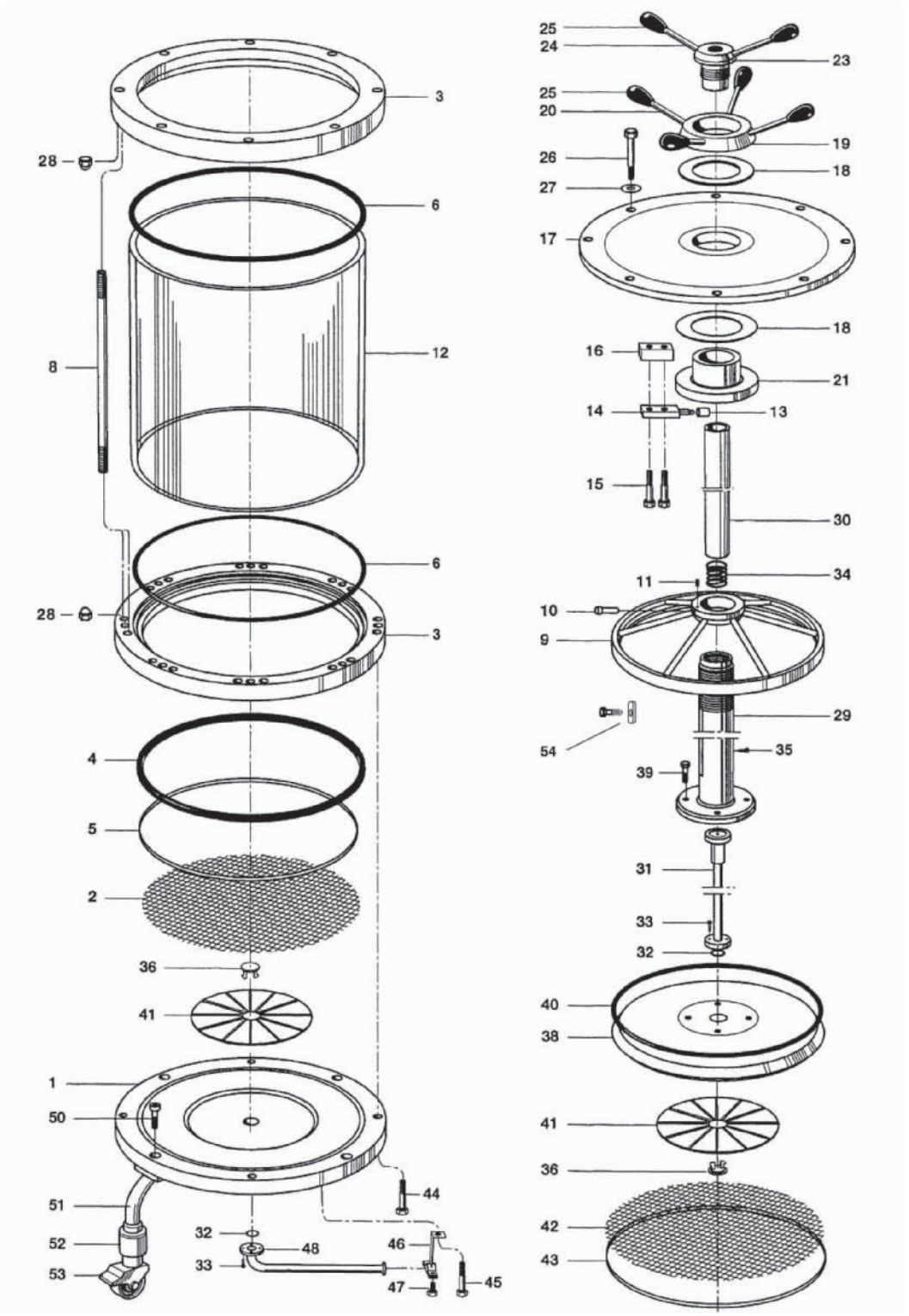

Fig. A-5. BPG 450 column parts identification.

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